U.S. DEPARTMENT OF COMMERCE
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PB-280 959

Source Assessment: Textile Plant Wastewater Toxics Study, Phase I

Monsanto Research Corp, Dayton, Ohio

Prepared for

Industrial Environmental Research Lab, Research Triangle Park, N C

Mar 78

PB 280 959

EPA-600/2-78-004h
March 1978

Environmental Protection Technology Series

SOURCE ASSESSMENT:
TEXTILE PLANT WASTEWATER TOXICS STUDY
PHASE I

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U.S. Environmental Protection Agency
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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/2-78-004h	2.	3. RECIPIENT'S ACCESSION NO. PB280959
4. TITLE AND SUBTITLE SOURCE ASSESSMENT: Textile Plant Wastewater Toxics Study--Phase I		5. REPORT DATE March 1978
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Gary D. Rawlings		8. PERFORMING ORGANIZATION REPORT NO. MRC-DA-774
9. PERFORMING ORGANIZATION NAME AND ADDRESS Monsanto Research Corporation 1515 Nicholas Road Dayton, Ohio 45407		10. PROGRAM ELEMENT NO. 1AB015; ROAP 21AXM-071
		11. CONTRACT/GRANT NO. 68-02-1874
12. SPONSORING AGENCY NAME AND ADDRESS EPA, Office of Research and Development Industrial Environmental Research Laboratory Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED Task Final; 1-12/77
		14. SPONSORING AGENCY CODE EPA/600/13

15. SUPPLEMENTARY NOTES IERL-RTP task officer is Max Samfield, Mail Drop 62, 919/541-2547. Earlier reports are in the EPA-600/2-76-032 and EPA-600/2-77-107 series.

16. ABSTRACT

The report gives results of the first phase of a study to provide chemical and toxicological baseline data on wastewater samples collected from textile plants in the U.S. Raw waste and secondary effluent wastewater samples were analyzed for 129 consent decree priority pollutants, effluent guidelines criteria pollutants, and nutrients. Level 1 chemical analyses were also performed. Secondary effluent samples from the 23 plants selected for study in the EPA/ATMI BATEA Study (American Textile Manufacturers Institute/best available technology economically achievable) (EPA Grant 804329) were submitted for the following bioassays: mutagenicity, cytotoxicity, clonal assay, freshwater ecology series (fathead minnows, Daphnia, and algae), marine ecology series (sheepshead minnows, grass shrimp, and algae), 14-day rat acute toxicity, and soil microcosm. The bioassay results indicated that 10 of the 23 textile plants have secondary effluents sufficiently toxic to proceed to a second phase of the study. In the second phase, samples will be collected from these 10 plants to determine the level of toxicity removal attained by selected tertiary treatment technologies.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group	
Pollution	Ecology	Pollution Control	13B	06F
Textile Industry		Stationary Sources	11E	
Waste Water		Toxic Materials		
Toxicity		Source Assessment	06T	
Bioassay		Baseline Data	06A	
Mutagens		Cytotoxicity	06E	
		Clonal Assay		

18. DISTRIBUTION STATEMENT Unlimited	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 169
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE A08 - A01

EPA Form 2220-1 (9-73)

EPA-600/2-78-004h
March 1978

SOURCE ASSESSMENT:
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PHASE I

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Contract No. 68-02-1874
ROAP No. 21AXM-071
Program Element No. 1AB015

EPA Task Officer: Max Samfield

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Prepared for

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Washington, DC 20460

PREFACE

The Industrial Environmental Research Laboratory (IERL) of the U.S. Environmental Protection Agency (EPA) has the responsibility for insuring that pollution control technology is available for stationary sources to meet the requirements of the Clean Air Act, the Federal Water Pollution Control Act, and solid waste legislation. If control technology is unavailable, inadequate, or uneconomical, then financial support is provided for the development of the needed control techniques for industrial and extractive process industries. The Chemical Processes Branch of the Industrial Processes Division of IERL has the responsibility for investing tax dollars in programs to develop control technology for a large number of operations (more than 500) in the chemical industries.

Monsanto Research Corporation (MRC) has contracted with EPA to investigate the environmental impact of various industries which represent sources of pollution in accordance with EPA's responsibility as outlined above. Dr. Robert C. Binning serves as MRC Program Manager in this overall program entitled "Source Assessment," which includes the investigation of sources in each of four categories: combustion, organic materials, inorganic materials, and open sources. Dr. Dale A. Denny of the Industrial Processes Division at Research Triangle Park serves as EPA Project Officer. Reports prepared in this program are of three types: Source Assessment Documents, State-of-the-Art Reports, and Special Project Reports.

Source Assessment Documents contain data on emissions from specific industries. Such data are gathered from the literature, government agencies, and cooperating companies. Sampling and analysis are also performed by the contractor when the available information does not adequately characterize the source emissions. These documents contain all of the information necessary for IERL to decide whether emissions reduction is required.

State-of-the-Art Reports include data on emissions from specific industries which are also gathered from the literature, government agencies, and cooperating companies. However, no extensive sampling is conducted by the contractor for such industries. Results from such studies are published as State-of-the-Art Reports for potential utility by the government, industry, and others having specific needs and interests.

Special projects provide specific information or services which are applicable to a number of source types or have special utility to EPA but are not part of a particular source assessment study. This special project report, "Source Assessment: Textile Plant Wastewater Toxics Study, Phase 1," was prepared to provide chemical and toxicological data on wastewater samples collected from selected textile plants in the United States. Dr. Max Samfield of the Chemical Processes Branch at IERL-RTP served as EPA Task Officer.

A second phase of this project is underway to collect samples of secondary effluents from 10 textile plants to determine the level of toxicity removal attained by selected tertiary treatment technologies.

ABSTRACT

The purpose of this study was to provide chemical and toxicological baseline data on wastewater samples collected from textile plants in the United States. Raw waste and secondary effluent wastewater samples were analyzed for 129 consent decree priority pollutants, effluent guidelines criteria pollutants, and nutrients; Level 1 chemical analyses were also performed. Secondary effluent samples from the 23 plants selected for study in the EPA/ATMI BATEA Study (American Textile Manufacturers Institute/best available technology economically achievable) (Grant No. 804329) were submitted for the following bioassays: mutagenicity, cytotoxicity, clonal assay, freshwater ecology series (fathead minnows, *Daphnia*, and algae), marine ecology series (sheepshead minnows, grass shrimp, and algae), 14-day rat acute toxicity, and soil microcosm. Since this was a screening study, samples of the textile plant intake water were not collected for chemical analysis.

Based on the bioassay results, 10 of the 23 textile plants were found to have secondary effluents sufficiently toxic to proceed to a second phase of the study. In the second phase, samples will be collected from these 10 plants to determine the level of toxicity removal attained by selected tertiary treatment technologies.

This report was submitted in partial fulfillment of Contract 68-02-1874 by Monsanto Research Corporation under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from January 1977 to December 1977.

CONTENTS

Preface. iii
Abstract v
Figures. ix
Tables xi
Abbreviations. xiv
Acknowledgments. xv

1. Introduction. 1
2. Summary 3
3. Results and Recommendations 12
4. Scope of Work 15
    Background 15
    Program objective. 19
    Project organization 20
5. Sampling Procedures 26
    Collection technique 26
    Sample container preparation 28
    Sampling logistics 28
    Sample shipping procedures 30
6. Wastewater Chemical Analyses. 31
    Effluent guidelines criteria pollutants. 31
    Analysis protocol for the 129 consent decree priority pollutants. 34
    Level 1 chemical analysis. 48
7. Bioassay of Secondary Effluents 83
    Microbiological mutagenicity 84
    Cytotoxicity assay 92
    Freshwater ecology toxicity. 98
    Marine ecology toxicity. 104
    Range-finding acute toxicity 14-day rat test . . . 111
    Soil microcosm test. 113
8. Discussion of Results 116
    Plant ranking by relative wastewater toxicity. . . 116
    Program outline for Phase II study 118

References 120
Appendices

A. Recommended list of priority pollutants 122
B. Priority pollutant analysis fractions 128
C. Addresses of persons associated with this textile study 131

CONTENTS (continued)

D. Reaction of fathead minnows and daphnia to textile secondary effluents 134
E. Reactions of rats to textile plant secondary effluent. 141
F. Protocol to test effects of waste materials on microbial respiration (carbon dioxide reduction) in a simple soil microcosm. 149

Glossary 150
Conversion Factors and Metric Prefixes 152

FIGURES

Number		Page
1	Program outline for Phase I: technology assessment for the ATMI/EPA Grant Study.	16
2.	Seven tertiary treatment modes for "best available technology" evaluation.	17
3	Overall program approach to determine BATEA	20
4	Program outline for Phase I of the MRC wastewater toxicity study.	21
5	Program outline for Phase II of the MRC wastewater toxicity study.	21
6	Sampling locations for Phase II of the MRC wastewater toxicity study.	22
7	Interpretation of bioassay test results	22
8	Scope of work for the analysis of textile plant wastewaters	24
9	EPA-recommended bioassay testing scheme for toxicity analysis of water samples.	24
10	Laboratories and persons involved in sample analysis of textile plant effluents	25
11	Phase I sampling locations.	26
12	MRC sample bottle label	30
13	Analytical scheme for volatile organics analysis. . .	36
14	Sample processing scheme for nonvolatile organics analysis.	36
15	Sample processing scheme for pesticide and PCB analysis.	38
16	EPA-recommended field handling scheme for liquid/slurry samples.	54
17	MRC-modified Level 1 field sampling and analysis scheme.	56
18	Level 1 organic analysis scheme	57
19	EPA, IERL/RTP modification to Level 1 organics analysis procedure.	59

FIGURES (continued)

Number		Page
20	Laboratories and EPA technical advisors involved in biotesting of effluent samples	86
21	Results of CHO-Kl clonal assay.	98
22	Example of graphically interpolated 24-hr, 48-hr, and 96-hr LC_{50}'s for juvenile sheepshead minnows exposed to textile effluent W.	109

TABLES

Number		Page
1	Summary of Priority Pollutants Found in Raw Waste Samples, from 129 Total, Showing Concentration Ranges and Number of Plants Where the Species were Identified	5
2	Summary of Priority Pollutants Found in Effluent Samples, from 129 Total, Showing Concentration Ranges and Number of Plants Where the Species were Identified	6
3	Summary of Metal, Criteria Pollutant, and Nutrient Analyses	7
4	Purpose of Selected Bioassary Tests in Evaluating the Potential Toxicity of Secondary Effluents .	8
5	Summary of Biotest Data for Secondary Effluent Wastewater Samples.	10
6	Chemical Compounds as Listed in the Consent Decree.	18
7	Sample Collection and Handling Requirements . . .	27
8	Portion of the Field Instructional Form Used by MRC to Assure Accurate Sample Collection and Preservation.	29
9	Analysis of Wastewater Samples for Effluent Guidelines Criteria Pollutants.	32
10	Summary of Continuous Samples and Volatile Organic Blank Analyses.	39
11	Textile Sample Extraction Dates	40
12	GC/MS Analyses for Base/Neutral Organic Compounds in Raw Waste and Effluent Samples	41
13	GC/MS Analyses for Volatile, Acid, Pesticide, and PCB Organic Compounds in Raw Waste and Effluent Samples	43
14	Number of Priority Organic Pollutants Found in the Raw Waste and Secondary Effluent Streams. .	47
15	Occurrence of Priority Organic Pollutants Combined from Raw Waste and Secondary Effluent Samples	49

TABLES (continued)

Number		Page
16	Consent Decree Metals Concentrations in Wastewater Samples	50
17	Other Metals Given by ICAP Analysis	52
18	Lower Detection Limit of Metals Analysis Systems.	54
19	Selected Parameters of Textile Effluent Samples from the Inorganic Segment of Level 1 Chemical Analysis Protocol	60
20	Field Analysis of Selected Secondary Wastewater Parameters on Filtered, Unextracted Samples as per Level 1 Analysis Protocol	61
21	Level 1 Spark Source Mass Spectrometer Metals Analysis of the Suspended Solids Collected on the Filter Paper During Field Filtration. . . .	62
22	Level 1 Spark Source Mass Spectrometers Metals Analysis of Filtered Secondary Effluent	67
23	Concentration of Methylene Chloride Extractable Organics in Filtered Secondary Effluents. . . .	72
24	Level 1 Infrared Analysis of the Organic Extracts	73
25	Level 1 Low Resolution Mass Spectrometer Analysis of Organic Fractions.	78
26	Bioassay Tests Used to Evaluate the Toxicity of Secondary Effluents	85
27	Estimated EC_{20} and EC_{50} Values for Cytotoxicity Screening of Filtered Secondary Effluent Samples	95
28	CHO-K1 Clonal Cytotoxicity Test	97
29	Dilutions Used for Freshwater Algal Tests	99
30	Nutrient Analysis of Secondary Effluent Samples .	100
31	Results of Freshwater Algae Bioassay Tests. . . .	100
32	Relative Ranking of Textile Plants by Tioxic and Stimulatory Effects of Secondary Wastewater on *S. Capricornutum*.	102
33	Acute Toxicity Data for Fathead Minnow and *Daphnia Pulex*	103
34	Results of Marine Algae Acute Toxicity Tests. . .	105

TABLES (continued)

Number — Page

35 Physical Description of Effluent Samples as They Arrived at BMRL for Sheepshead Minnow and Grass Shrimp Acute Toxicity Analysis 107

36 Acute Toxicity of 14 Textile Effluents to Juvenile Sheepshead Minnows. 109

37 Acute Toxicity of 14 Textile Effluents to Grass Shrimp 110

38 Physical Examinations in Acute Toxicity in Rodents. 112

39 Results of Soil Microcosm Tests on Secondary Wastewater Samples 115

40 Relative Toxicity Ranking by Bioassay Test 116

41 Prioritization of Textile Plants by Toxicity of Secondary Effluent. 118

42 Sample Schedule at Each of the 10 Textile Plants 119

ABBREVIATIONS

ATMI -- American Textile Manufacturers Institute
ATP -- adenosine triphosphate
BATEA -- best available technology economically achievable
BOD$_5$ -- 5-day biochemical oxygen demand
COD -- chemical oxygen demand
DO -- dissolved oxygen
EC -- electron capture detector on a gas chromatograph
EC$_{50}$ -- effective concentration at which 50% of the test species reach the desired effect
GC -- gas chromatograph
GCMA -- gas chromatography mass analysis
ICAP -- inductively coupled argon plasma
IR -- infrared analyzer
LC -- liquid chromatography
LC$_{50}$ -- lethal concentration which causes 50% mortality in the test species
LD$_{50}$ -- lethal dosage which causes 50% mortality in the test species
LRMS -- low resolution mass spectrometer
MS -- mass spectrometer
PCB -- polychlorinated biphenyls
ppt -- parts per thousand
RAM -- rabbit alvelor macraphage
SSMS -- spark source mass spectrometer
T/C -- ratio of population density in treated samples to population density in controls
TDS -- total dissolved solids
TKN -- total Kjeldahl nitrogen
TOC -- total organic carbon
TSS -- total suspended solids
v/v -- volume-to-volume ratio
w/v -- weight-to-volume ratio

ACKNOWLEDGMENTS

The author would like to express his gratitude to members of the American Textile Manufacturers Institute (ATMI), Carpet and Rug Institute (CRI), and Northern Textile Association (NTA) for their extensive cooperation in this project.

The author would also like to thank Dr. Max Samfield, EPA Project Leader on this task, for his guidance and project coordination.

SECTION 1

INTRODUCTION

The Industrial Environmental Research Laboratory - RTP (IERL-RTP) of the U.S. Environmental Protection Agency (EPA) is currently engaged in a joint study with the American Textile Manufacturers Institute (ATMI) (EPA Grant No. 804329) to determine the best available technology economically achievable (BATEA) for textile plant wastewaters. A total of 23 textile mills representing eight textile processing categories and having well-operated secondary wastewater treatment facilities were selected by EPA and ATMI for the BATEA study. For that study, two mobile pilot plants containing four tertiary wastewater treatment technologies were constructed to gather technical data to identify the best available technology applicable to the 23 plants. Two additional tertiary treatment technologies were tested in the laboratory. The grant study focused on only a limited number of so-called criteria pollutants; i.e., 5-day biochemical oxygen demand, chemical oxygen demand, color, sulfides, total suspended solids, phenol, and pH.

However, on 7 June 1976 the U.S. District Court of Washington, D.C., issued a consent decree (resulting from Natural Resources Defense Council et al. *v*. Train) requiring EPA to enhance development of effluent standards. The court mandate focused federal water pollution control efforts on potentially toxic and hazardous pollutants. In response to the consent decree EPA developed a list of 129 specific compounds (known as priority pollutants) that the agency agreed to consider during the standards setting process. Based on the consent decree, EPA-IERL/RTP decided to conduct a study parallel to the ATMI/EPA Grant Study of the textile industry. The objective of the IERL/RTP study was to determine both the removal efficiencies for the 129 consent decree priority pollutants and the reduction in toxicity by the six tertiary treatment technologies being investigated under the original grant study.

The overall wastewater toxicity study is divided into two phases. The first, covered by this report, establishes a baseline data base concerning toxicity and level of priority pollutants present in raw wastewater and secondary effluents at 23 textile plants. These data are used to screen the 23 plants and to select those plants with secondary effluents of highest toxicity for further study. Toxicity tests were designed to evaluate only the reduction in wastewater toxicity by control technologies, not the potential environmental impacts on receiving waters.

The second phase of the effort, to be covered in a subsequent report, will determine the reduction in priority pollutant concentrations and in toxicity by the mobile pilot plant tertiary treatment systems. Only those plants selected in the first phase of the study will be investigated.

Covering the first phase of the toxics study, this report describes sampling, chemical analysis, and bioassay procedures used to establish baseline data. Chemical analyses of raw waste and secondary effluents are presented for the 23 basic plants and for 9 additional textile plants. Bioassay data are presented for secondary effluents from the basic 23 plants.

The plants are ranked according to relative secondary effluent toxicity, and a number of plants are selected for study in the second phase of the overall program. Modifications and recommendations for improvements to the sampling, chemical analysis, and bioassay protocols are also discussed.

SECTION 2

SUMMARY

The purpose of this Phase I study was to provide chemical and toxicological baseline data on wastewater samples collected from selected textile plants in the United States. Raw waste (untreated wastewater) and secondary effluent (wastewater from secondary wastewater treatment facilities) samples were collected from the 23 textile plants selected for the ATMI/EPA Grant Study (Grant No. 804329) and from 9 additional textile plants. Since this was a screening study, samples of the textile plant intake water were not collected for chemical analysis.

Samples were analyzed for 129 consent decree priority pollutants and for effluent guidelines criteria pollutants. The Level 1 chemical analytical scheme developed by EPA-IERL/RTP was employed to detect other possible pollutants. Nutrient levels were measured at 23 of the 32 plants to supplement interpretation of algal bioassays. The following bioassays were performed on secondary effluent samples from the 23 plants chosen for the ATMI/EPA Grant Study: mutagenicity, cytotoxicity, clonal assay, freshwater ecology series (fathead minnows, *Daphnia*, and algae), marine ecology series (sheepshead minnows, grass shrimp, and algae), 14-day rat acute toxicity, and soil microcosm.

Grab samples and 8-hr continuous samples were collected both before and after the wastewater treatment system at each of the 32 plants. Samples were stored in ice at 4°C and shipped by air freight to the laboratories for analysis. Chemical analyses and bioassays were performed at eight EPA and commercial laboratories

Analysis for the 129 priority pollutants in raw waste and secondary effluent samples (totaling 64 samples) was performed by Monsanto Research Corporation (MRC). Analytical procedures followed those recommended by EPA. However, the recommended analytical protocol for priority pollutant analysis is still in the developmental stage and requires further verification and validation. Consequently, the analytical results of textile wastewater samples must be looked upon as good estimates of which priority pollutants are present, with concentrations accurate to within an order of magnitude.

The EPA analytical protocol divided the 129 priority pollutants into 5 fractions for analysis: volatile compounds, base/neutral compounds, acid compounds, pesticides and polychlorinated biphenyls (PCB), and metals. EPA recommended that laboratories not acquire analytical standards for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) because of its extreme toxicity. Asbestos was not analyzed due to the presence of interfering fibrous materials in textile wastewaters.

A summary of the organic compounds found in the 32 raw waste and 32 secondary effluent samples is given in Tables 1 and 2. Of the 114 organic compounds on the priority pollutant list, a total of 45 different compounds were found, 39 in raw waste samples and 34 in secondary effluent samples. On an individual plant basis the greatest number of organic compounds found in a raw waste and in a secondary effluent sample were 14 and 8, respectively, with an average number per plant of 7 in the raw waste and 5 in the secondary effluent. The predominant compounds were bis(2-ethylhexyl) phthalate in 54 samples (0.5 mg/m^3 to 300 mg/m^3), toluene in 44 samples (0.4 mg/m^3 to 300 mg/m^3), and ethylbenzene in 30 samples (0.7 mg/m^3 to 3,000 mg/m^3).

A summary of the 13 priority pollutant metals and cyanide concentrations in raw waste and secondary effluent samples is given in Table 3, which also summarizes the criteria pollutant and nutrient concentrations for secondary effluent samples. Nutrient analyses were performed to support freshwater algae bioassays.

On an individual plant basis it was frequently observed, especially for the metals data, that the concentration of a specific pollutant was greater in the secondary effluent sample than in the raw waste sample. This phenomenon is due, in part, to the hydraulic retention time of the wastewater treatment facility. Since raw waste and secondary effluent samples were collected simultaneously, concentrations in the secondary effluent were due to raw waste loads that entered the treatment system 1 day to 30 days prior to sampling. The average retention time for the 32 plants was about 5 days.

Level 1 chemical analyses were performed on secondary effluent samples from 15 of the 23 basic textile plants. Level 1 protocol identifies classes of compounds present in environmental samples and measures the general concentration range. Results indicate that total concentration of methylene chloride extractable organics ranges from 3 g/m^3 to 64 g/m^3. This value is 5 to 10 times lower than the range for total organic carbon (Table 3).

In the Level 1 procedure each sample was fractionated by a liquid chromatography column into eight fractions based on polarity. Infrared analysis of each fraction indicated the presence of aliphatic hydrocarbons, esters and acids, aromatic compounds, phthalate esters, and fatty acid groups. Low resolution mass spectrophotometric analysis of the eight fractions of each sample detected the following types of compounds: paraffinic/olefinic,

TABLE 1. SUMMARY OF PRIORITY POLLUTANTS FOUND IN RAW WASTE SAMPLES, FROM 129 TOTAL, SHOWING CONCENTRATION RANGES AND NUMBER OF PLANTS WHERE THE SPECIES WERE IDENTIFIED

Volatile organic			Base/neutral organic		
Compound	Number of times found	Concentration range, mg/m^3	Compound	Number of times found	Concentration range, mg/m^3
Toluene	22	2 to 300	Naphthalene	20	0.03 to 300
Benzene	4	5 to 200	Dimethyl phthalate	5	3 to 110
Chloroform	12	2 to 500	Diethyl phthalate	12	0.2 to 70
Chlorobenzene	6	1 to 300	Bis(2-ethylhexyl) phthalate	27	0.5 to 300
Ethylbenzene	20	0.7 to 2,800	1,4-Dichlorobenzene	5	1 to 210
Trichlorofluoromethane	2	30 to 50	1,2,4-Trichlorobenzene	8	30 to 440
1,1,1-Trichloroethane	5	2 to 300	1,2-Dichlorobenzene	8	0.1 to 300
Trichloroethylene	8	2 to 200	Anthracene	1	0.1
1,1,2,2-Tetrachloroethylene	8	15 to 1,100	Pyrene	1	0.9
Trans-1,2,-dichloroethylene	1	2	Acenaphthene	7	9 to 270
1,1-Dichloroethane	2	0.6 to 4	Di-n-butyl phthalate	6	2 to 23
1,2-Dichloropropane	1	2	Fluorene	2	5 to 15
Cis-1,3-dichloropropene	1	2	Hexachlorobenzene	2	0.5 to 2
			N-Nitrosodiphenylamine	1	11
			2,6-Dinitrotoluene	1	50
			Indeno(1,2,3-cd)pyrene	1	2
Acid organic			**Pesticide**		
Phenol	19	0.5 to 100	β-BHC	1	0.4
Phentachlorophenol	8	2 to 70	Heptachlor	1	6
2-Nitrophenol	1	70			
ρ-Chloro-*m*-cresol	1	5			
4-Nitrophenol	1	70			
2,4,6-Trichlorophenol	2	0.7 to 20			
2-Chlorophenol	1	130			

TABLE 2. SUMMARY OF PRIORITY POLLUTANTS FOUND IN EFFLUENT SAMPLES, FROM 129 TOTAL, SHOWING CONCENTRATION RANGES AND NUMBER OF PLANTS WHERE THE SPECIES WERE IDENTIFIED

Volatile organic			Base/neutral organic		
Compound	Number of times found	Concentration range, mg/m^3	Compound	Number of times found	Concentration range, mg/m^3
Toluene	22	0.4 to 110	Naphthalene	5	0.5 to 250
Benzene	2	0.5 to 60	Dimethyl phthalate	3	0.2 to 1
Chloroform	5	5 to 60	Diethyl phthalate	9	0.5 to 10
Chlorobenzene	2	4 to 30	Bis(2-ethylhexyl)phthalate	27	2 to 230
Ethylbenzene	10	0.7 to 3,000	1,4-Dichlorobenzene	3	0.05 to 2
Trichlorofluoromethane	6	2 to 2,100	1,2,4-Trichlorobenzene	6	2 to 920
Trichloroethylene	2	5 to 80	1,2-Dichlorobenzene	5	0.2 to 25
1,1,2,2-Tetrachloroethylene	3	0.4 to 40	Anthracene	1	4
Cis-1,3-dichloropropene	1	6	N-Nitroso-di-n-propylamine	2	2 to 20
Trans-1,3-dichloropropene	2	0.9 to 4	Pyrene	4	0.1 to 0.3
Bromodichloromethane	1	2	Acenaphthene	2	0.5 to 2
			Di-n-butyl phthalate	3	4 to 60
			Hexachlorobenzene	3	0.3 to 0.8
			Butylbenzyl phthalate	1	70
Acid organic			Pesticides		
Phenol	2	2 to 3	α-BHC	1	0.3
2,4-Dimethylphenol	2	8 to 9	Heptachlor	1	2
p-chloro-*m*-cresol	1	2			
2,4,6-Trichlorophenol	1	20			
Chloro cresol	1	30			
2-Chlorophenol	1	10			

TABLE 3. SUMMARY OF METAL, CRITERIA POLLUTANT, AND NUTRIENT ANALYSES

Metal			Criteria pollutant[a]		Nutrient[a]	
	Concentration range, g/m^3					
Element	Raw waste sample	Secondary effluent sample	Pollutant	Concentration range, g/m^3	Parameter	Concentration range, g/m^3
Antimony	0.0005 to 0.06	0.0005 to 0.07	BOD_5[b]	<5 to 170	Nitrite	0 to 17
Arsenic	0.005 to 0.2	0.005 to 0.02	COD[c]	45 to 1,600	Nitrate	0.002 to 40
Beryllium	<0.0001	<0.0001	Color (APHA)[d]	10 to 2,500	Ammonia	0.02 to 14
Cadmium	0.0005 to 0.05	0.0005 to 0.01	Sulfide	0.01 to 6	TKN[e]	2 to 40
Chromium	0.0002 to 0.9	0.0002 to 2.0	Phenol	0.01 to 0.2	*o*-Phosphate	0.02 to 11
Copper	0.0002 to 2.4	0.0002 to 0.3	TSS[f]	0.02 to 580	Total phosphorus	0.4 to 15
Cyanide	0.004 to 0.2	0.004 to 0.2	pH	5.8 to 10	TOC[g]	19 to 260
Lead	0.001 to 0.2	0.001 to 0.2				
Mercury	0.0005 to 0.004	0.0005 to 0.0009				
Nickel	0.01 to 0.2	0.01 to 0.2				
Selenium	<0.005	<0.005				
Silver	0.005 to 0.1	0.005 to 0.1				
Thallium	<0.005	<0.005				
Zinc	0.03 to 8.0	0.07 to 38				

[a] For secondary effluent samples.

[b] 5-day biochemical oxygen demand.

[c] Chemical oxygen demand.

[d] American Public Health Association color standards.

[e] Total Kjehldehl nitrogen.

[f] Total suspended solids.

[g] Total organic carbon.

bis(hydroxy-t-butyl phenol) propane, tri-t-butyl benzene, alkyl phenols, dichloroaniline, toluene-sulfonyl groups, vinyl stearate and azo compounds.

Bioassays used were selected by EPA and include tests for assessment of both health and ecological effects. Health effects tests estimate the potential mutagenicity, potential or presumptive carcinogenicity, and potential toxicity of the samples to mammalian organisms. Ecological effects tests focus on the potential toxicity of the samples to vertebrates (fish), invertebrates (daphnids and shrimp), and plants (algae) in freshwater, marine and terrestrial ecosystems.

A total of 8 bioassay systems were tested using 21 different tester organisms to evaluate the toxicity of secondary effluents. Table 4 lists the bioassays used and the purpose of each test.

TABLE 4. PURPOSE OF SELECTED BIOASSAY TESTS IN EVALUATING THE POTENTIAL TOXICITY OF SECONDARY EFFLUENTS

Bioassay system	Test organism	Purpose of test
Microbial mutagenicity	Nine different strains of bacteria and one of yeast.	To determine if a chemical mutagen (possibly a carcinogen) is present. These microbial strains were selected because of their sensitivity to various classes of chemical compounds.
Cytotoxicity	Rabbit alveolar cells and Chinese hamster ovary cells.	To measure metabolic impairment and death in mammalian cells. These primary cell cultures have some degree of metabolic repair capability.
Freshwater and marine static bioassay	Fathead minnow, daphnids, sheepshead minnows, and grass shrimp.	To detect potential toxicity to organisms present in aquatic environments.
Freshwater and marine algal assay	Freshwater and marine algae.	To detect potential growth inhibition and stimulation effects on aquatic plants.
Range finding acute toxicity	Young adult rats.	Whole animal test to detect potential toxic effects to mammals. These live animals were selected because of the extensive data base on their response to known chemicals and because they have several metabolic systems closely approximating those in humans.
Terrestrial ecology	Soil microorganisms.	To determine potential inhibition and stimulation effects on soil microorganisms. These data are useful if the effluent is used for crop irrigation.

A summary of the bioassay results is presented in Table 5. Toxicity is expressed as the percent of a secondary effluent solution that will cause the effect specified for each bioassay over the testing period. For the cytotoxicity, *Daphnia* and algal bioassays an Effective Concentration 20 or 50 (EC_{20} or EC_{50}) was calculated. EC_{20} for the cytotoxicity test means the concentration of secondary effluent which impairs metabolic processes in 20% of the test cells.

The viability test is a measure of the cells' ability to survive exposure to the sample, and the adenosine triphosphate (ATP) test measures the quantity of the coenzyme ATP produced, indirectly measuring cellular metabolic activity.

EC_{50} for the algal tests means the concentration of secondary effluent which causes a 50% reduction in algal growth as compared to a control sample. The freshwater algae test was performed over a 14-day period and the marine algae test over a 96-hr period.

For the fathead minnow, sheepshead minnow, and grass shrimp bioassays, death was used to measure toxicity, which was expressed as Lethal Concentration 50 (LC_{50}). LC_{50} indicates the calculated concentration of secondary effluent that is expected to cause the death of 50% of the test species. Since rats were given a specific quantity of secondary effluent, toxicity was expressed as Lethal Dose 50 (LD_{50}). LD_{50} indicates the quantity of material fed to the rats that resulted in the death of 50% of the test animals.

The measure of toxicity to a soil microcosm was the quantity of carbon dioxide (CO_2) produced after sample exposure as compared to a control sample. The quantity of CO_2 produced over a 3-wk period after subtracting the quantity produced by the control was plotted on graph paper. The slope of the curve then represented the rate of increase or decrease in CO_2 production due to addition to the sample.

Based on the bioassay results, the EPA Bioassay Subcommittee ranked the 23 plants according to their overall secondary effluent toxicity. The following nine textile plants were selected for further study based on their relatively high ranking: N, A, L, T, C, P, S, V, and W. Note the low toxicity response for Plant Y where the effluent samples were collected after the polishing pond.

During the second phase of this program the secondary effluents from these plants will be treated using the mobile pilot plants constructed under the ATMI/EPA Grant Study. Since effluent samples were inadvertently collected between the aeration lagoon and settling basin at Plant R, it will also be included in the Phase II program. While each of these 10 plants is being tested

TABLE 5. SUMMARY OF BIOTEST DATA FOR SECONDARY EFFLUENT WASTEWATER SAMPLES[a,b]

	Cytotoxicity		Freshwater ecology series			Marine ecology series			
Plant	Viability (24-hr EC_{20}), % secondary effluent	ATP (24-hr EC_{20}), % secondary effluent	Fathead minnow (96-hr LC_{50}), % secondary effluent	*Daphnia* (48-hr EC_{50}), % secondary effluent	Algae[c] (14-day EC_{20}), % secondary effluent	Sheepshead minnow (96-hr LC_{50}), % secondary effluent	Grass shrimp (96-hr LC_{50}), % secondary effluent	Algae[c] (96-hr EC_{50}), % secondary effluent	Soil microsm, normalized relative CO_2 rate change[d]
A	NAT[e]	NAT	19.0	9.0	76	62.0	21.2	-[f]	-0.032
B	NAT	NAT	NAT	NAT	30	NAT	NAT	-[g]	0.020
C	16.8[h]	6.1[h]	46.5	41.0	0[i]	69.5	12.8	90	-0.005
D	NAT	NAT	NAT	NAT	0[i]	-[f]	-[f]	-[f]	-0.099
E	NAT	NAT	NAT	7.8	2[j]	NAT	NAT	10 to 50	-0.048
F	NAT	9.4	NAT	81.7	0[i]	NAT	NAT	85	-0.039
G	NAT	NAT	64.7	62.4	0[i]	NAT	NAT	59	0.017
H	NAT	NAT	-[k]	40% dead at 100% concentration	96	-[f]	-[f]	-[f]	-0.083
J	NAT	NAT	NAT	NAT	0[i]	-[f]	-[f]	-[f]	-0.163
K	NAT	NAT	NAT	NAT	0[i]	NAT	NAT	77	-0.004
L	NAT	4.0	23.5	28.0	42	NAT	NAT	1.7	-0.020
M	NAT	NAT	NAT	60.0	0[i]	-[f]	-[f]	-[f]	-0.059
N	13.3	3.8	48.8	100% dead at all dilutions	2[k]	47.5	26.3	2.3	0.059
P[l]	NAT	NAT	NAT	NAT	43	-[f]	-[f]	9.0	0.022
R	NAT	NAT	16.5	8.0	93	-[f]	-[f]	-[f]	-0.062
S	-[f]	-[f]	NAT	NSA[m]	0[i]	NAT	NAT	-[g]	-0.017
T	NAT	2.5	46.5	NAT	0[i]	68.0	34.5	70	0.020
U	NAT	NAT	NAT	12.1	0[i]	NAT	NAT	-[g]	0.055
V	NAT	NAT	36.0	9.4	0[i]	-[f]	-[f]	94	-0.066
W	NAT	13.7	55.2	6.3	94	37.5	19.6	50	0.031
X	NAT	4.8	NAT	NAT	0[i]	NAT	NAT	-[g]	0.047
Y	NAT	NAT	NAT	NAT	0[i]	-[f]	-[f]	-[f]	-0.172
Z	NAT	NAT	NAT	42.6	18	-[f]	-[f]	-[f]	-0.112

[a] No chemical mutagen was detected by the 10 microbial strains.

[b] No rat mortality after 14 days due to maximum dosage of 10^{-5} m^3/kg body weight (LD_{50}). However, six samples (B, F, L, N, and S) showed potential body weight effects, and sample R resulted in eye irritation.

[c] Effect was algal growth inhibition.

[d] Negative sign indicates inhibition in CO_2 generation rate compared to a control sample; positive number indicates CO_2 stimulation.

[e] No acute toxicity.

[f] Analysis not performed on this sample.

[g] Growth inhibition <50% in 100% solution of secondary effluent.

[h] pH = 9.1 not adjusted before testing.

[i] Sample stimulated algal growth.

[j] 95% growth inhibition in 2% solution of secondary effluent.

[k] Diseased batch of fish nullified this analysis.

[l] Sample inadvertently collected prior to settling pond.

[m] No statistical analysis because heavy solids concentration obscured the analysis; the sample did not appear to be acutely toxic.

to determine BATEA, samples will also be collected from the "best" treatment system to evaluate the reduction in acute toxicity. Samples will also be collected to measure removal efficiencies for the 129 priority pollutants. Textile plant intake water samples will also be collected and analyzed for the priority pollutants.

SECTION 3

RESULTS AND RECOMMENDATIONS

Several results can be noted from the data presented with respect to textile plant secondary effluents.

1. None of the secondary effluent samples resulted in a positive mutagenic response or indicated acute toxicity to rats.

2. Even though the series of bioassays are unrelated in terms of toxicity mechanisms, the data did provide sufficient information to relatively rank secondary effluents in terms of toxicity and to select those plants for further study under the tertiary treatment technology assessment.

3. The effluent sample collected from Plant R was inadvertently collected between the aeration lagoon and settling basin. Therefore the sample tested was that of the aeration lagoon slurry. Note that for bioassays where the sample was first filtered (cytotoxicity and freshwater algae), the toxicity was slight; for unfiltered samples (fathead minnow, daphnia, and soil microcosm) the toxic effects were significant. This result tends to imply that the toxicity for Plant R is associated with the filterable solids. Plant R will be resampled in Phase II of the program to further evaluate the toxicity response.

4. In terms of priority pollutants, only 56 of the 114 organic species were detected in either raw waste or secondary effluent sample, with 49 found in the raw waste samples and 46 in the secondary effluent samples. The dominant organic species present in secondary effluent samples include common plasticizer species, such as phthalates, and common raw materials used by the textile manufacturing industry, such as chlorobenzenes.

5. Of the 13 priority pollutant metals, beryllium, selenium, and thallium were below analytical detection limits in all raw waste and secondary effluent samples. The dominant metal species detected were arsenic, chromium, copper, and zinc.

6. The data indicates that secondary treatment by aeration lagoons and clarifiers produce a significant decrease in phenolic compounds and toluene and a moderate reduction in chlorobenzenes.

7. It is difficult to accurately evaluate the treatment efficiencies because for this program raw waste and secondary effluent samples were collected simultaneously. Due to the 1-day to 30-day hydraulic retention time in the treatment plants, secondary effluent samples reflect the waste loading 1-day to 30-day in the past. Also, data are not available to indicate the quantity of organic compounds stripped from the wastewater due to the action of surface aerators.

As a result of performing newly developing bioassay tests, priority pollutant analyses, and Level 1 chemical analyses on environmental samples, several recommendations can be made with respect to improvements. The major recommendations are discussed below:

1. For bioassay screening purposes, it proved to be more economical to conduct mutagenicity and rat acute toxicity tests using the maximum dose. If no toxicity was indicated, then the test was completed; when toxicity was detected, then the dose response testing scheme was used.

2. Toxicity testing should be performed on filtered and unfiltered samples. Also, due to the detection limits of the mutagenicity, cytotoxicity, and rat acute toxicity tests, consideration should be given to testing concentrated samples.

3. Whenever extractions are performed on environmental samples, the percent of extractable organics should be determined. For the Level 1 chemical analysis scheme, potentially more organics can be extracted in methylene chloride if the sample pH is adjusted to pH 10 or 11 as opposed to use of neutral pH. Since the Level 1 chemical analysis scheme requires field extractions, a presurvey and preliminary tests should be performed to determine the extractability of the sample and to identify potential problems.

4. For priority pollutant volatiles analysis, MRC obtained better results by cryogenic trapping at -40°C than at room temperature as recommended by EPA. In addition, to reduce interference effects, three internal standards were used as opposed to the one recommended.

5. To identify the source of priority pollutants in wastewater samples, samples of the intake water to the industry complex should be analyzed.

SECTION 4

SCOPE OF WORK

BACKGROUND

To understand the nature and purpose of the textile wastewater toxics program it is first necessary to briefly review the events which formed the study's foundation. The principal event occurred on 1 October 1974 when the American Textile Manufacturers Institute (ATMI) filed a petition with the U.S. Fourth Circuit Court of Appeals asking for review of the 1983 effluent guidelines proposed for the textile industry. ATMI's grounds for the suit were that the best available technology economically achievable (BATEA) had not been demonstrated for the textile industry. As a result, ATMI and EPA filed a joint motion for delay of the petition, stating that additional information would be developed through a cooperative study by ATMI and EPA (IERL/RTP).

The objective of this ATMI/EPA Grant Study was to gather enough technical and economic data to determine what is the BATEA for removing criteria pollutants from textile wastewaters. Criteria pollutants for the textile industry include 5-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), color, sulfide, pH, chromium, phenol, and total suspended solids (TSS). On 3 January 1975 the court instructed ATMI and EPA to proceed as promptly as feasible to a completion and review of the study.

The ATMI/EPA Grant Study was divided into two phases: Phase I, to determine the best available technology, and Phase II, to determine which technology(s) was economically achievable. A generalized program outline of Phase I is shown in Figure 1. To evaluate the best available technology, two mobile pilot plants were constructed by ATMI. This strategy allowed for real-time, flowthrough treatment studies. Each pilot plant contained four tertiary wastewater treatment unit operations; one was scheduled to visit 12 textile plants and the other to visit 11. Two additional tertiary treatment technologies were laboratory tested.

Treatment operations in each mobile unit include a reactor/clarifier (using combinations of alum, lime, ferric chloride, and anionic and cationic polyelectrolytes), two multimedia filters, three granular activated carbon columns, ozonation and dissolved air flotation. Powdered activated carbon treatability tests

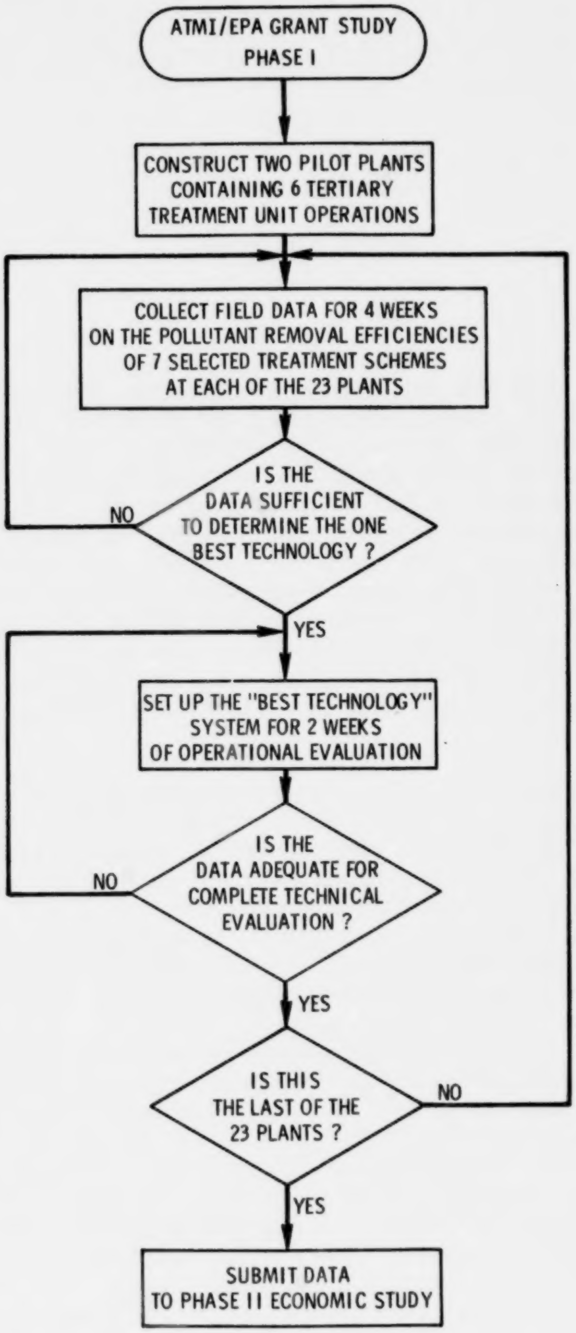
ATMI/EPA GRANT STUDY
PHASE I
CONSTRUCT TWO PILOT PLANTS CONTAINING 6 TERTIARY TREATMENT UNIT OPERATIONS
COLLECT FIELD DATA FOR 4 WEEKS ON THE POLLUTANT REMOVAL EFFICIENCIES OF 7 SELECTED TREATMENT SCHEMES AT EACH OF THE 23 PLANTS
IS THE DATA SUFFICIENT TO DETERMINE THE ONE BEST TECHNOLOGY ?
NO
YES
SET UP THE "BEST TECHNOLOGY" SYSTEM FOR 2 WEEKS OF OPERATIONAL EVALUATION
IS THE DATA ADEQUATE FOR COMPLETE TECHNICAL EVALUATION ?
NO
YES
IS THIS THE LAST OF THE 23 PLANTS ?
NO
YES
SUBMIT DATA TO PHASE II ECONOMIC STUDY

Figure 1. Program outline for Phase I: technology assessment for the ATMI/EPA Grant Study.

were performed in the laboratory instead of in the field with the pilot plant. Using these six unit operations ATMI and EPA selected seven treatment systems for evaluation (Figure 2).

MODE A :	REACTOR / CLARIFIER → MULTIMEDIA FILTER
MODE B :	MULTIMEDIA FILTER → GRANULAR ACTIVATED CARBON COLUMNS
MODE C :	MULTIMEDIA FILTER → OZONATOR
MODE D :	OZONATOR
MODE E : (OPTIONAL)	REACTOR / CLARIFIER → MULTIMEDIA FILTER → GRANULAR ACTIVATED CARBON → OZONATOR
MODE F :	COAGULATION → MULTIMEDIA FILTER
MODE G :	DISSOLVED AIR FLOTATION

Figure 2. Seven tertiary treatment modes for "best available technology" evaluation.

Each of the seven treatability systems was to be set up, and operational and pollutant data were to be collected over a 2-day to 3-day period. Based on that data, the "best" system was to be selected and set up for 2 weeks of operational evaluation. These data were then to be forwarded to Phase II for economic evaluation.

The second event that formed the foundation for this project occurred when a group of environmental action organizations filed a class action suit against EPA (Natural Resources Defense Council et al. *v*. Train, U.S. District Court of Washington, D.C.) for not developing and promulgating regulations establishing effluent limitations and guidelines and new source performance standards for 21 industrial point sources, including the textile industry. As a result, on 7 June 1976 the court issued a consent decree requiring EPA to enhance development of effluent standards.

The most significant result from the court mandate was that it focused federal water pollution control efforts on potentially toxic and hazardous pollutants. The original consent decree required that 38 classes of chemical compounds (Table 6) be analyzed in wastewater samples. Recognizing the difficulty of analyzing for all chemical species present in each category of compounds, EPA developed a list of 129 specific compounds (Appendix A) representative of the classes of compounds listed in the consent decree. These compounds are referred to as the consent decree priority pollutants, or priority pollutants for short.

TABLE 6. CHEMICAL COMPOUNDS AS LISTED IN THE CONSENT DECREE

Acenaphthene
Acrolein
Acrylonitrile
Aldrin/Dieldrin
Antimony and compounds
Arsenic and compounds
Asbestos
Benzene
Benzidine
Beryllium and compounds
Cadmium and compounds
Carbon tetrachloride
Chlordane (technical mixture and metabolites)
Chlorinated benzenes (other than dichlorobenzenes)
Chlorinated ethanes (including 1,2-dichloroethane, 1,1,1-trichloroethane, and hexachloroethane)
Chloroalkyl ethers (chloromethyl, chloroethyl, and mixed ethers)
Chlorinated naphthalene
Chlorinated phenols (other than those listed elsewhere; includes trichlorophenols and chlorinated cresols)
Chloroform
2-Chlorophenol
Chromium and compounds
Copper and compounds
Cyanides
DDT and metabolites
Dichlorobenzenes (1,2-,1,3-, and 1,4-dichlorobenzenes)
Dichlorobenzidine
Polychlorinated biphenyls (PCB)
Polynuclear aromatic hydrocarbons (including benzanthracenes, benzopyrenes, benzofluoranthene, chrysenes, dibenzanthracenes, and indenopyrenes)
Selenium and compounds
Silver and compounds
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)
Tetrachloroethylene
Thallium and compounds
Toluene
Toxaphene
Trichloroethylene
Vinyl chloride
Zinc and compounds

EPA also developed a sampling and analytical procedures manual to be used as a laboratory guide for the analysis of priority pollutants (1). The analytical methods recommended by EPA are still in the developmental phase and require further verification and validation.

Therefore, in addition to evaluating the removal of criteria pollutants by tertiary treatment technologies, EPA was charged with the task of evaluating the removals of toxicity and priority pollutants by the treatment systems.

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(1) Draft Final Report: Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants. U.S. Environmental Protection Agency, Cincinnati, Ohio, March 1977. 145 pp.

The final event which influenced the formation of the present program was the three-phase sampling and analytical strategy for environmental assessment developed by EPA, Process Measurements Branch, IERL/RTP. The purpose of the assessment procedure was to determine in a stepwise and cost-effective manner all chemical species being discharged to the environment from a point source. Level 1, the first part of the three-phase approach, is designed to focus available resources on emissions that have a high potential for causing measurable health or ecological effects.

The second phase, Level 2, has as its goal the identification and quantification of specific compounds. Level 3 is designed to continuously monitor indicator compounds as surrogates for a large number of specific pollutants. At the start of this textile project, only Level 1 analytical and biological procedures were available (2).

In addition to chemical analyses, the Level 1 recommended protocol included bioassay testing procedures for evaluating toxicity removal by control technologies (3). Bioassays are required to provide direct evidence of complex biological effects such as synergism, antagonism, and bioavailability.

PROGRAM OBJECTIVE

The fundamental objective of the textile wastewaters program conducted by MRC in conjunction with the EPA is to determine the reduction in toxicity and priority pollutant concentrations achieved by the tertiary treatment technologies under investigation in the ATMI/EPA Grant Study. The latter study focuses directly on the treatability of criteria pollutants. Thus, the overall EPA-IERL/RTP textile program consists of two separate projects, each with different activities, running parallel in time, but converging towards the same goal: determination of the best available technology economically achievable for removing textile wastewaters (Figure 3).

To evaluate the reduction in toxicity in a cost-effective manner for the MRC/EPA project, a two-phase approach was developed. Phase I was designed to collect baseline toxicity data on

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(2) Hamersma, J. W., S. L. Reynolds, and R. F. Maddalone. IERL-RTP Procedures Manual: Level 1 Environmental Assessment. EPA-600/2-76-160a (PB 257 850), U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, June 1976. 147 pp.

(3) Duke, K. M., M. E. Davis, and A. J. Dennis. IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests for Pilot Studies. EPA-600/7-77-043 (PB 268 484), U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, April 1977. 114 pp.

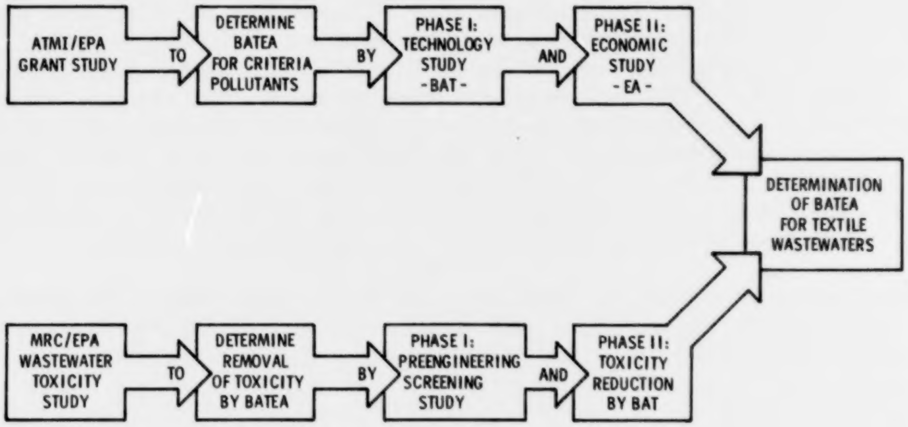
ATMI/EPA GRANT STUDY
TO
DETERMINE BATEA FOR CRITERIA POLLUTANTS
BY
PHASE I: TECHNOLOGY STUDY -BAT-
AND
PHASE II: ECONOMIC STUDY -EA-
DETERMINATION OF BATEA FOR TEXTILE WASTEWATERS
MRC/EPA WASTEWATER TOXICITY STUDY
TO
DETERMINE REMOVAL OF TOXICITY BY BATEA
BY
PHASE I: PREENGINEERING SCREENING STUDY
AND
PHASE II: TOXICITY REDUCTION BY BAT

Figure 3. Overall program approach to determine BATEA.

secondary effluents from 23 selected textile plants and to rank the plants in descending order of toxicity (Figure 4). Phase II was designed to determine the level of toxicity removal attained by the tertiary treatment systems in the ATMI/EPA Grant Study at only those plants with relatively high secondary effluent toxicity (Figure 5). Sampling locations for Phase II of the study are shown in Figure 6, and the strategy used for evaluating control technologies in terms of toxicity removal is illustrated in Figure 7.

PROJECT ORGANIZATION

The major effort of the Phase I MRC/EPA screening study was devoted to the collection, chemical analysis, and biological toxicity testing of single, 8-hr composited wastewater samples from the 23 textile plants scheduled for testing in the ATMI/EPA Grant Study. In addition, samples were collected from nine other textile plants for chemical analyses only. Wastewater characterization data were therefore assembled for a total of 32 plants.

The scope of work for Phase I was divided into three separate task areas, each based on different EPA data requirements, as shown in Figure 8. CPB (Chemical Processes Branch, IERL/RTP, Project Officer, M. Samfield) requested chemical and bioassay data on secondary effluent samples from the 23 textile plants scheduled to be studied in the ATMI/EPA Grant Study. These data were used to characterize and compare the relative toxicities of the plant effluents tested. EGD (Effluent Guidelines Division, EPA, Washington, J. D. Gallup) requested chemical analyses of the raw waste streams entering the 23 wastewater treatment plants, as well as chemical analyses of the raw waste and secondary effluent streams at 9 additional textile plants. Raw waste and effluent data were needed to evaluate the pollutant removal efficiencies

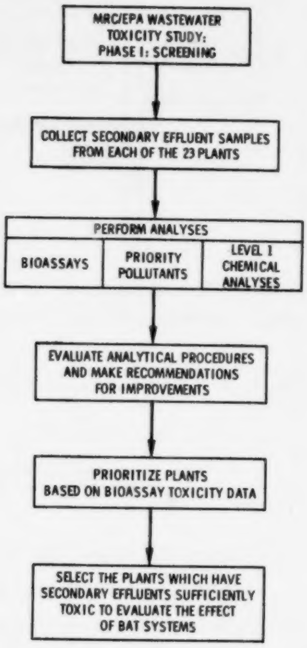
MRC/EPA WASTEWATER TOXICITY STUDY: PHASE I: SCREENING
COLLECT SECONDARY EFFLUENT SAMPLES FROM EACH OF THE 23 PLANTS
PERFORM ANALYSES
BIOASSAYS
PRIORITY POLLUTANTS
LEVEL 1 CHEMICAL ANALYSES
EVALUATE ANALYTICAL PROCEDURES AND MAKE RECOMMENDATIONS FOR IMPROVEMENTS
PRIORITIZE PLANTS BASED ON BIOASSAY TOXICITY DATA
SELECT THE PLANTS WHICH HAVE SECONDARY EFFLUENTS SUFFICIENTLY TOXIC TO EVALUATE THE EFFECT OF BAT SYSTEMS

Figure 4. Program outline for Phase I of the MRC wastewater toxicity study.

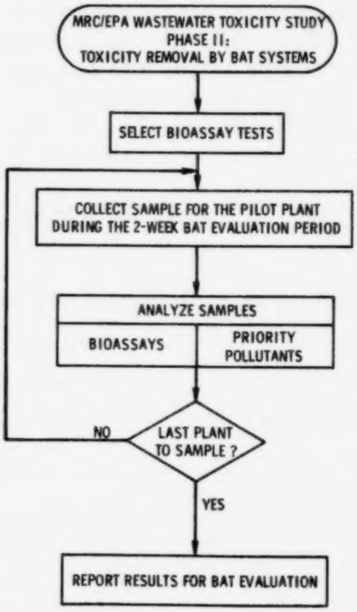
MRC/EPA WASTEWATER TOXICITY STUDY PHASE II: TOXICITY REMOVAL BY BAT SYSTEMS
SELECT BIOASSAY TESTS
COLLECT SAMPLE FOR THE PILOT PLANT DURING THE 2-WEEK BAT EVALUATION PERIOD
ANALYZE SAMPLES
BIOASSAYS
PRIORITY POLLUTANTS
LAST PLANT TO SAMPLE ?
NO
YES
REPORT RESULTS FOR BAT EVALUATION

Figure 5. Program outline for Phase II of the MRC wastewater toxicity study.

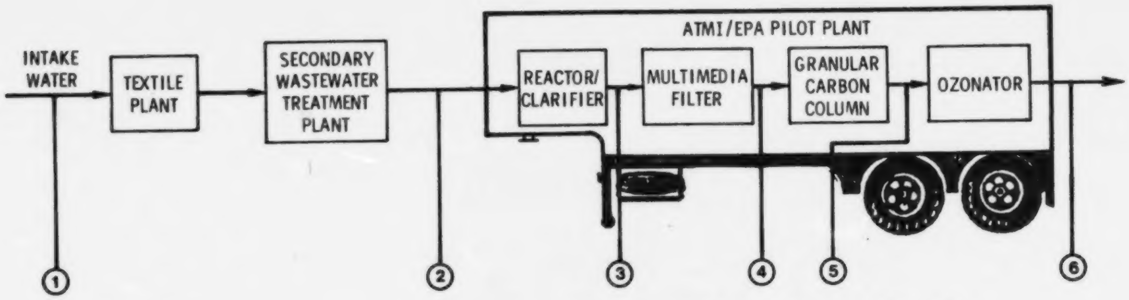
ATMI/EPA PILOT PLANT
INTAKE WATER
TEXTILE PLANT
SECONDARY WASTEWATER TREATMENT PLANT
REACTOR/ CLARIFIER
MULTIMEDIA FILTER
GRANULAR CARBON COLUMN
OZONATOR
①
②
③
④
⑤
⑥

Figure 6. Sampling locations for Phase II of the MRC wastewater toxicity study.

INTERPRETATION OF BIOASSAY TEST RESULTS

Bioassay Results		Toxic Substance Interpretation
Inlet	Outlet	
+	+	Control Technology Is Not Effective
+	-	Control Technology Is Effective
-	+	Control Technology Is Deterimental
-	-	Control Technology Is Not Deterimental

Figure 7. Intepretation of bioassay test results.

of current state-of-the-art secondary treatment systems. In order to detect other possible pollutant species, PMB (Process Measurements Branch, IERL/RTP, L. D. Johnson) requested that Level 1 chemical characterization also be performed on the effluent samples at the basic 23 textile plants (2). Since these data were requested after the program began, only 15 textile plants were sampled for Level 1 chemical characterization.

Chemical characterization of wastewater samples involves four categories of analysis:

- 129 consent decree priority pollutants analysis (1)
- nutrient analysis (4, 5)
- effluent guidelines criteria pollutants analysis (4, 5)
- Level 1 chemical characterization (2)

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(4) Manual of Methods for Chemical Analysis of Water and Wastes. EPA-625/6-76-003a (PB 259 973), U.S. Environmental Protection Agency, Cincinnati, Ohio, 1976. 317 pp.

(5) Standard Methods for the Examination of Water and Wastewater, Fourteenth Edition. American Public Health Association, Washington, D.C., 1976. 874 pp.

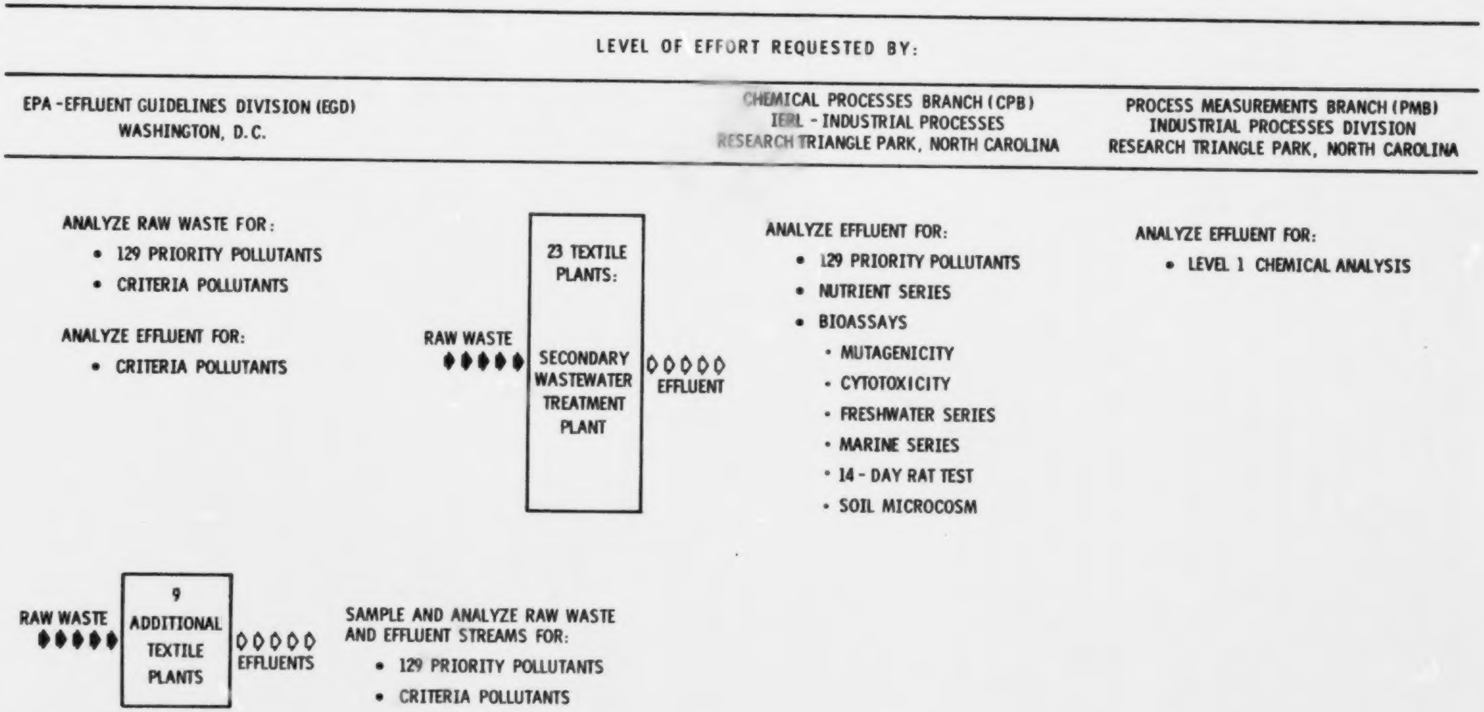

LEVEL OF EFFORT REQUESTED BY:
EPA - EFFLUENT GUIDELINES DIVISION (EGD)
WASHINGTON, D.C.
CHEMICAL PROCESSES BRANCH (CPB)
IERL - INDUSTRIAL PROCESSES
RESEARCH TRIANGLE PARK, NORTH CAROLINA
PROCESS MEASUREMENTS BRANCH (PMB)
INDUSTRIAL PROCESSES DIVISION
RESEARCH TRIANGLE PARK, NORTH CAROLINA
ANALYZE RAW WASTE FOR:
• 129 PRIORITY POLLUTANTS
• CRITERIA POLLUTANTS
ANALYZE EFFLUENT FOR:
• CRITERIA POLLUTANTS
RAW WASTE
23 TEXTILE PLANTS:
SECONDARY WASTEWATER TREATMENT PLANT
EFFLUENT
ANALYZE EFFLUENT FOR:
• 129 PRIORITY POLLUTANTS
• NUTRIENT SERIES
• BIOASSAYS
· MUTAGENICITY
· CYTOTOXICITY
· FRESHWATER SERIES
· MARINE SERIES
· 14 - DAY RAT TEST
· SOIL MICROCOSM
ANALYZE EFFLUENT FOR:
• LEVEL 1 CHEMICAL ANALYSIS
RAW WASTE
9 ADDITIONAL TEXTILE PLANTS
EFFLUENTS
SAMPLE AND ANALYZE RAW WASTE AND EFFLUENT STREAMS FOR:
• 129 PRIORITY POLLUTANTS
• CRITERIA POLLUTANTS

Figure 8. Scope of work for the analysis of textile plant wastewaters.

The 129 consent decree priority pollutants as listed in Appendix A are divided into volatile compounds, nonvolatile compounds, and metals (see Appendix B). The nutrient series required to support algal tests includes analysis of nitrite, nitrate, ammonia, total Kjeldahl nitrogen (TKN), *o*-phosphate, phosphorus, and total organic carbon (TOC). Effluent guidelines criteria pollutants include 5-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), sulfide, color, pH, total suspended solids (TSS), total dissolved solids (TDS), and total phenol. A detailed description of Level 1 chemical characterization is given in Section 6.

The bioassay scheme established by EPA for evaluating the reduction in toxicity of water samples by control technologies is shown in Figure 9. All the tests shown were used in this project. The marine ecology series and the soil microcosm tests were requested after the project began, therefore data were obtained from 15 textile plants as opposed to the basic 23 plants.

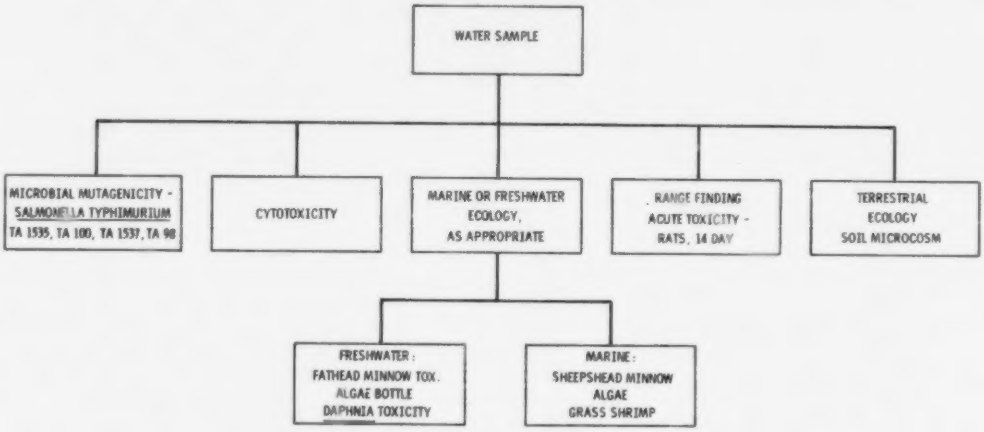

WATER SAMPLE
MICROBIAL MUTAGENICITY - SALMONELLA TYPHIMURIUM TA 1535, TA 100, TA 1537, TA 98
CYTOTOXICITY
MARINE OR FRESHWATER ECOLOGY, AS APPROPRIATE
RANGE FINDING ACUTE TOXICITY - RATS, 14 DAY
TERRESTRIAL ECOLOGY SOIL MICROCOSM
FRESHWATER: FATHEAD MINNOW TOX. ALGAE BOTTLE DAPHNIA TOXICITY
MARINE: SHEEPSHEAD MINNOW ALGAE GRASS SHRIMP

Figure 9. EPA-recommended bioassay testing scheme for toxicity analysis of water samples.

Figure 10 illustrates the distribution of samples among the eight EPA and private laboratories that performed the chemical analyses and bioassay tests. Appendix C lists the names and addresses of all persons involved in the textile project.

MRC collected raw wastes and effluent samples at 23 of the ATMI/EPA-designated plants. Wastewater samples were collected by EPA-Environmental Research Laboratory (ERL) (Athens, Georgia) at two of the additional textile plants and sent to MRC for chemical analysis. Sverdrup and Parcel and Associates, Inc., (St. Louis, Missouri) collected the remaining samples at the additional seven plants and sent them to MRC for chemical analysis.

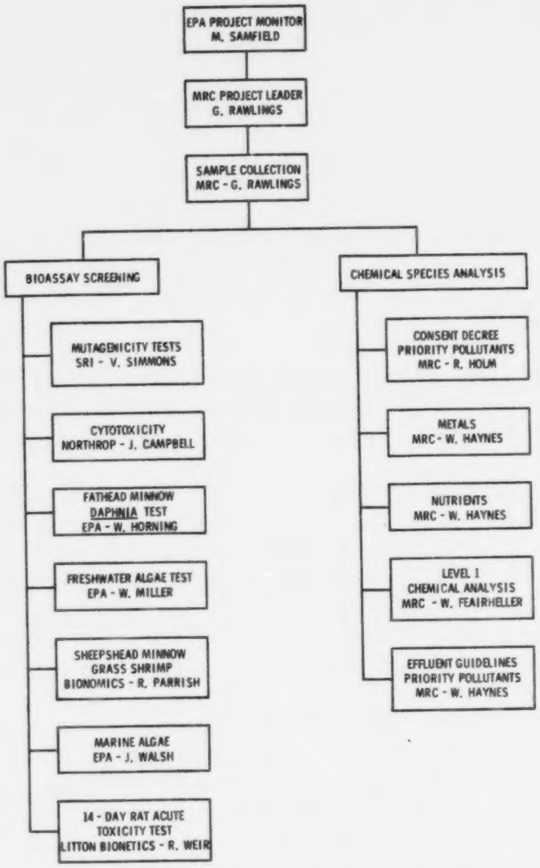

EPA PROJECT MONITOR
M. SAMFIELD
MRC PROJECT LEADER
G. RAWLINGS
SAMPLE COLLECTION
MRC - G. RAWLINGS
BIOASSAY SCREENING
MUTAGENICITY TESTS
SRI - V. SIMMONS
CYTOTOXICITY
NORTHROP - J. CAMPBELL
FATHEAD MINNOW
DAPHNIA TEST
EPA - W. HORNING
FRESHWATER ALGAE TEST
EPA - W. MILLER
SHEEPSHEAD MINNOW
GRASS SHRIMP
BIONOMICS - R. PARRISH
MARINE ALGAE
EPA - J. WALSH
14 - DAY RAT ACUTE
TOXICITY TEST
LITTON BIONETICS - R. WEIR
CHEMICAL SPECIES ANALYSIS
CONSENT DECREE
PRIORITY POLLUTANTS
MRC - R. HOLM
METALS
MRC - W. HAYNES
NUTRIENTS
MRC - W. HAYNES
LEVEL 1
CHEMICAL ANALYSIS
MRC - W. FEAIRHELLER
EFFLUENT GUIDELINES
PRIORITY POLLUTANTS
MRC - W. HAYNES

Figure 10. Laboratories and persons involved in sample analysis of textile plant effluents.

Sampling, analytical, and bioassay procedures followed those recommended in EPA reports (1-5). All procedural modifications instituted to accommodate the textile wastewater samples are discussed in detail in the remaining sections of this report.

SECTION 5

SAMPLING PROCEDURES

COLLECTION TECHNIQUE

Wastewater was collected by composite and grab sampling techniques. Composite samplers (Isco Model 1680) were used to collect raw waste samples for analysis of nonvolatile organics and metals.

Tygon® sample tubing used was washed with detergent, rinsed thoroughly, and given a final washing with organic-free water. A 0.001-m^3 sample blank was then collected and analyzed for organic leachates. Organic-free water was prepared by passing water, distilled in glass, through a 0.6-m-long activated carbon column. The blank was collected in glass, sealed with a Teflon®-lined cap, and stored in ice at 4°C until analyzed.

Grab sampling techniques were used to collect raw waste samples for other analyses, and for all secondary effluent samples, Figure 11. Eight individual grab samples were collected at equally spaced time intervals during the normal working day. To insure that each of the eight laboratories received a sufficient portion of the same sample, grab samples were collected in a Teflon-lined, 0.01-m^3 stainless steel bucket. A specified aliquot was transferred to each of the sample bottles from this container. Care was taken to insure that the sample remained homogeneous throughout each of the 10-min pouring sessions. Containers for volatile organics analysis were collected and sealed first to minimize possible evaporation losses.

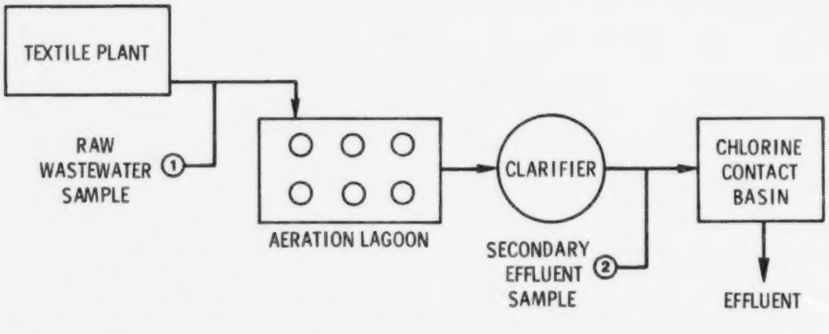


Figure 11. Phase I sampling locations.

TABLE 7. SAMPLE COLLECTION AND HANDLING REQUIREMENTS (4)

Analysis	Total sample vol required, m^3	Container used	Preservation steps	Storage temper-ature, C
Consent decree pollutants				
Volatile organics	320×10^{-6} [a,b]	Glass	_[c]	4[d]
Nonvolatile organics	$4,000 \times 10^{-6}$	Glass		4
Metals	$1,000 \times 10^{-6}$	Plastic	Nitric acid 5×10^{-3} m^3/m^3	4
Nutrient analysis				
Total organic carbon	25×10^{-6}	Glass	Sulfuric acid to pH < 2	4
Ammonia	400×10^{-6}	Glass	Sulfuric acid to pH < 2	4
Total Kjeldahl nitrogen	500×10^{-6}	Glass	Sulfuric acid to pH < 2	4
Nitrate	100×10^{-6}	Glass	Sulfuric acid to pH < 2	4
Nitrite	50×10^{-6}	Glass		
Orthophosphate	50×10^{-6}	Glass		
Total phosphorus	50×10^{-6}	Glass		
Criteria pollutants				
Biochemical 5-day oxygen demand	$1,000 \times 10^{-6}$	Glass		4
Chemical oxygen demand	50×10^{-6}	Glass	Sulfuric acid to pH <2	4
Color	50×10^{-6}	Glass		4
Sulfide	500×10^{-6}	Glass	2×10^{-6} m^3 zinc acetate	4
Total suspended solids	100×10^{-6}	Glass		
Phenol	500×10^{-6}	Glass	Phosphoric acid to pH < 4, 10^3 g copper sulfate/m^3	4
Cyanide	500×10^{-6}	Plastic	Sodium hydroxide to pH > 12	4
Freshwater ecology				
Fathead minnow and *Daphnia*	$60,000 \times 10^{-6}$	Glass		4
Algae	$9,600 \times 10^{-6}$	Plastic		4
Marine ecology				
Sheepshead minnow and grass shrimp	$60,000 \times 10^{-6}$	Glass		4
Algae	$2,000 \times 10^{-6}$	Plastic		4
Soil microcosm	100×10^{-6}	Plastic		4
Ames test	250×10^{-6}	Glass		4
Cytotoxicity tests	250×10^{-6}	Glass		4
14-day Rat test	$1,000 \times 10^{-6}$	Glass		4

[a] Eight individual 40×10^{-6} m^3 samples

[b] 10^{-6} m^3 equals 1 ml.

[c] Blanks indicate no chemical preservation required.

[d] If free chlorine is present in the sample, 3×10^{-3} m^3 of 10% solution of sodium thiosulfate is added to each of the 4×10^{-5} m^3 sample vials to neutralize the residual chlorine; otherwise, abnormally high trihalomethane values may result.

An additional 0.02 m^3 were required to properly apportion samples into the six 0.02-m^3 bottles for the marine and freshwater ecology tests. Samples were poured into these bottles at the end of each of the eight grab sampling sessions. There was no visible change in flow rate during each of the 15-min sample collection periods. Fluctuations in effluent and raw waste stream flow rates were usually on a 45-min to 1-hr time schedule.

SAMPLE CONTAINER PREPARATION

All glass containers, except the 0.02-m^3 bottles, were thoroughly cleaned with strong acid (50% sulfuric acid + 50% nitric acid), rinsed, and heated in a glass annealing oven at 400°C for at least 30 min. The 0.02-m^3 bottles were detergent washed and thoroughly rinsed. The glass containers used to store samples for mutagenicity testing were rinsed with acetone. The rest of the glass containers were rinsed with methylene chloride and dried in the oven at 100°C. All glass bottles had Teflon-lined caps.

Plastic sample containers were thoroughly cleaned before use. Each bottle was washed with detergent and tap water, then rinsed with 1:1 nitric acid/tap water, 1:1 hydrochloric acid/tap water, and, finally, deionized distilled water (1).

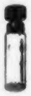
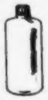
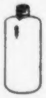
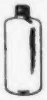
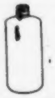
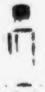
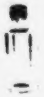
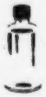
SAMPLING LOGISTICS

The type and volume of sample container varied, depending on the analysis to be made. Some samples required the addition of chemical preservatives in the field to prevent deterioration during shipment to the laboratory. The volume of sample required, the container used to hold the sample, and the preservation steps used in this project are shown in Table 7 (4).

A field sampling instructional worksheet was designed to facilitate the arduous task of filling 37 glass and plastic bottles of different sizes requiring different sample volumes and preservatives at each plant. Table 8 shows part of this worksheet.

Each sampling day, before sampling, bottle labels were filled out and affixed to the appropriate sample bottles. Figure 12 shows the bottle label that MRC has designed for sample identification.

TABLE 8. PORTION OF THE FIELD INSTRUCTIONAL FORM USED BY MRC TO ASSURE ACCURATE SAMPLE COLLECTION AND PRESERVATION

	Organics								
Type of test	Volatile	Nonvolatile	Metals	TOC, NH_3, TKN COD, nitrate	Marine algae	Cyanide	Phenol	Sulfide	Ames test
Sample destination	MRC	MRC	MRC	MRC	Gulf Breeze	MRC	MRC	MRC	SRI
Bottle identification number	1	2	3	4	5	6	7	8	9
Number of bottles	8	1	1	1	1	1	1	1	1
Type of bottle									
Sample size 10^{-6} m^3	40	3,785	240	240	240	240	62	62	62
	Chemicals added for sample preservation								
Sample number 1	+3 x 10^{-6} m^3 Sodium thiosulfate, seal	None	+5 x 10^{-6} m^3 Nitric acid	+Sulfuric acid, pH <2	None	+4 x 10^{-6} m^3 Sodium hydroxide, check with potassium iodide paper	pH <4 with phosphoric acid	+2 x 10^{-6} m^3 Zinc acetate	None
2	Same as No. 1	None	None	Same as No. 1	None	None	0.5 g copper sulfate	None	None
3	Same as No. 1	None	None	Same as No. 1	None	None	None	None	None
4	Same as No. 1	None	None	Same as No. 1	None	None	Same as No. 1	None	Same
5	Same as No. 1	None	None	Same as No. 1	None	None	None	None	None
6	Same as No. 1	None	None	Same as No. 1	None	None	None	None	None
7	Same as No. 1	None	None	Same as No. 1	None	None	None	None	None
8	Same as No. 1	Seal	Seal	Same as No. 1	None	pH > 12, check seal	Same as No. 1	Seal	Seal

Job____

Sample or Run No.______________________________

Sample Location________________________________

Type of Sample_________________________________

Analyze for____________________________________

Preservation___________________________________

Comments_______________________________________

____

____

____

Log No.______________________ Date____________

Initials_______________________________________

Figure 12. MRC sample bottle label.

SAMPLE SHIPPING PROCEDURES

Each bottle was capped and sealed with tape to prevent leakage. Glass bottles were individually wrapped to prevent breakage.

Sample bottles were then packed in one-piece, molded, styrene foam shipping cartons with 3.8-cm walls and fitted tops. Each such unit was then placed in a corrugated cardboard box.

Each carton was half-filled with sample bottles, filled with ice, sealed with cellophane tape, and reinforced with 0.05-m duct tape.

Address labels were affixed to box tops. Warning labels--"This carton contains glass and ice"--"Hold at airport and call ____" messages were also put on the box tops.

All samples were shipped by conventional air freight on the day that they were collected. The airlines selected offered the most direct route without carrier changes.

SECTION 6

WASTEWATER CHEMICAL ANALYSES

EFFLUENT GUIDELINES CRITERIA POLLUTANTS

Parameters determined under the category of effluent guidelines criteria pollutants were: 5-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), color, sulfides, total suspended solids (TSS), pH, and total phenol. As sample shipments arrived at MRC, they were logged in and distributed to the designated technicians for analysis.

Analytical and supporting procedures followed those described in References 4 and 5.

Criteria pollutants were determined on the raw waste and secondary effluent streams from the basic 23 plants and the additional 9 plants.

Results of the chemical analyses are given in Table 9. The first row of numbers for each plant represents data obtained on the raw waste stream, and the second row of numbers corresponds to the wastewater treatment plant effluent stream. All values except color and pH are given in g/m^3 (ppm). Color was measured using the American Public Health Association (APHA) system.

Effluent values obtained from wastewater treatment facilities in some plants were greater than those of the influent raw waste. This occurred, in part, because the wastewater entered the treatment system 1 day to 5 days prior to leaving the treatment plant. The hydraulic retention time in textile wastewater treatment plants ranged from 1 day to 30 days, with an average value of 5 days.

All of the textile plants sampled had a secondary wastewater treatment facility that included a lagoon with several surface aerators, followed by a clarifier. Several plants used equalization basins prior to the aerated lagoons. Effluent samples were collected between the clarifier and the finishing pond in plants that had both. The two exceptions were plant Y, where the sample was taken after the finishing pond, and plant R, where the effluent sample was inadvertently collected between the aerated lagoon and the settling basin. All other plant effluent samples were collected after the clarifiers.

TABLE 9. ANALYSIS OF WASTEWATER SAMPLES FOR EFFLUENT GUIDELINES CRITERIA POLLUTANTS

	Criteria pollutant, g/m³							
Plant code	5-Day biochemical oxygen demand	Chemical oxygen demand	Color, APHA	Sulfide	Phenol	Total suspended solids	pH	Cyanide
A/raw waste	459	1,735	2,000	6.0	0.092	165	10.7	<0.004
A/effluent	168	1,652	2,000	4.0	0.065	228	7.3	0.015
B/raw waste	1,050	1,264	1,400	1.4	0.042	32	10.5	0.017
B/effluent	<5	99	90	0.2	0.015	8	7.5	<0.004
C/raw waste	445	802	2,600	5.2	0.074	49	11.2	0.007
C/effluent	25	396	1,920	5.0	0.088	300	10	0.013
D/raw waste	71	224	1,875	<0.02	0.024	16	10	0.21
D/effluent	6.6	64	1,625	2.8	0.018	154	7.2	0.21
E/raw waste	18	2,660	250	<1	0.069	52	10	<0.004
E/effluent	<5	78	30	<1	0.014	19	7.2	<0.004
F/raw waste	194	583	150	2.1	0.74	23	9.2	<0.004
F/effluent	69	276	80	0.1	0.028	44	7.4	<0.004
G/raw waste	203	1,340	300	<1	0.028	37	11	<0.004
G/effluent	42	502	300	<1	0.054	6	7.5	0.006
H/raw waste	288	320	1,250	<0.02	0.047	39	10	<0.004
H/effluent	14	300	500	<0.02	0.019	43	7.6	<0.004
J/raw waste	210	810	1,875	1.8	0.063	0.01	11	<0.004
J/effluent	25	376	1,375	1.8	0.024	0.023	7.8	<0.004
K/raw waste	564	1,725	40,000	<1	0.067	69	10	<0.004
K/effluent	<5	131	150	<1	0.018	21	7.2	<0.004
L/raw waste	379	1,117	1,300	4.5	0.038	19	7.4	<0.004
L/effluent	13	234	370	3.0	0.026	78	5.8	0.172
M/raw waste	830	2,265	1,000	<1	0.037	210	11	<0.004
M/effluent	<5	255	500	<1	0.025	21	7.5	<0.004
N/raw waste	334	1,140	1,050	1.1	0.156	68	9.2	<0.004
N/effluent	36	286	90	0.1	0.068	77	7.0	<0.004
P/raw waste	680	172	300	6.19	0.228	6	10	0.19
P/effluent	28	45	250	6.09	0.032	45	7.1	0.14
R/raw waste	450	1,692	1,500	<1	0.282	87	10	<0.004
R/effluent[a]	70	830	2,000	<1	0.162	225	8.1	<0.004
S/raw waste	219	559	250	9.2	0.107	25	10	0.007
S/effluent	59	1,035	75	<1	0.029	581	7.8	<0.004

(continued)

TABLE 9 (continued)

	Criteria pollutant, g/m^3							
Plant code	5-Day biochemical oxygen demand	Chemical oxygen demand	Color, APHA	Sulfide	Phenol	Total suspended solids	pH	Cyanide
T/raw waste	501	500	1,345	7.6	0.073	28	9.5	<0.004
T/effluent	32	414	350	6.0	0.041	35	7.4	<0.004
U/raw waste	400	1,464	3,200	5.6	0.057	111	10	<0.004
U/effluent	24	748	2,480	3.5	0.007	92	7.3	0.212
V/raw waste	53	b	500	<1	0.018	54	9.0	0.006
V/effluent	<5	128	500	<1	0.016	26	7.1	0.018
W/raw waste	1,920	6,124	2,200	0.5	0.670	2,300	10.4	0.015
W/effluent	84	837	1,900	0.1	0.232	300	8.1	0.020
X/raw waste	237	786	1,200	0.75	0.940	24	10.2	<0.004
X/effluent	15	258	10	0.01	0.035	18	7.2	0.101
Y/raw waste	122	457	10,000	<1	0.064	33	10.5	<0.004
Y/effluent	<5	115	250	<1	0.022	17	8.0	<0.004
Z/raw waste	351	812	500	2.48	0.56	20	10	<0.004
Z/effluent	<5	105	750	<1	0.023	13	8	<0.004
JJ/raw waste	b	1,545	b	<1	0.144	b	b	0.005
JJ/effluent	b	510	b	<1	0.055	b	b	0.028
KK/raw waste	b	1,955	b	<1	0.150	b	b	<0.004
KK/effluent	b	447	b	<1	0.052	b	b	<0.004
LL/raw waste	b	727	b	<1	0.001	b	b	0.008
LL/effluent	b	155	b	<1	0.094	b	b	0.006
MM-1	b	b	b	<1	0.033	b	b	<0.004
MM-2	b	b	b	<1	0.031	b	b	<0.004
MM-3	b	b	b	<1	0.036	b	b	<0.004
MM-4	b	b	b	<1	0.039	b	b	<0.004
NN/raw waste	b	938	b	<1	0.043	b	b	0.04
NN/effluent	b	236	b	<1	0.014	b	b	<0.004
OO/raw waste	b	1,889	b	<1	0.082	b	b	<0.004
OO/effluent	b	635	b	<1	0.026	b	b	<0.004
PP[c]	b	339	b	<1	0.044	b	b	<0.004
Y-001/raw waste	b	b	b	b	b	b	b	<0.004
Y-001/effluent	b	b	b	b	b	b	b	0.029
C-001[c]	b	b	b	b	b	b	b	<0.004

[a] Secondary effluent sample was inadvertently collected between the aerated lagoon and settling ponds.

[b] Analysis not performed on sample.

[c] Secondary effluent sample only.

ANALYSIS PROTOCOL FOR THE 129 CONSENT DECREE PRIORITY POLLUTANTS

Recommended analytical procedures (1) developed by EPA were used throughout this project. It is important to realize that these EPA procedures are still under development and require further verification and validation. Therefore, the data presented in this section only serve to identify which of the 129 chemical species are present and to indicate the general concentration ranges within an order of magnitude.

Adaptations of these procedures to accommodate the special requirements of textile wastewaters and/or any ambiguities in analytical techniques are discussed below. Three chemical species were not determined in this project: endrin aldehyde, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and asbestos. EPA-Environmental Monitoring and Support Laboratory (EMSL) recommended that TCDD should be omitted because of its extreme toxicity, and the potential health hazard involved in preparing standard solutions from the pure compound. Pure endrin aldehyde could not be obtained in time to prepare standard solutions. Asbestos was eliminated, as recommended by EPA-IERL-RTP and EPA-EGD, due to the presence of other fibrous materials in textile wastewaters.

The analytical protocol (2) divides the 129 chemical species into three basic categories: volatile organics, nonvolatile organics, and metals. Appendix B lists the chemical species which belong to each category. The following sections outline the analytical procedures and MRC modifications for each category.

Volatile Organics

The recommended analytical method was designed to determine those chemical species which are amenable to the Bellar purge and trap method. Eight 40 x 10^{-6}-m^3, hermetically sealed glass vials, stored in ice, were sent to the laboratory from each sampling site. The vials were composited within 1 day of receipt at the laboratory. Two vials of composite solution were sealed and retained at 4°C as reserve samples. Volatiles from 5 x 10^{-6}-m^3 samples of composite solution were sparged with helium onto two Tenax GC-silica-packed sample tubes. (Internal standards were added to the solutions in the later stages of the program. The majority of the samples had been sparged and stored before the protocol (1) was received and appropriate internal standard could be obtained.) The second Tenax tube was used as a backup sample. Tenax tubes were sealed under a nitrogen atmosphere in glass tubes and stored in a freezer at -18°C until analyzed.

Analyses were carried out using a Hewlett Packard 5981 GC-Mass Spectrometer with 5934 Data System. Sample tubes were heated to 180°C over a 1-min period and held at that temperature for 4 min to desorb the compounds onto a Carbowax 1500 column held at -40°C. Cryogenic trapping at -40°C (liquid nitrogen cooling) gave better reproducibility of retention time than using the suggested temperature of 30°C, for compounds with boiling points below room temperature. After desorption, the GC column temperature was raised 8°C/min to 170°C.

The mass spectrometric analysis method involves fragmentation of molecules using electron bombardment (70 eV). Masses and relative intensities of the most characteristic molecular fragments for each compound are listed in the protocol (1). The population of ion fragments covering the mass range from 35 atomic mass units to 500 atomic mass units was measured every 6 s, and the data were stored on magnetic tape.

These data allow the operator to reconstruct chromatograms of observed intensity for an individual mass during the course of the scanning. Specific molecules may be detected in the presence of other compounds by examining the reconstructed intensity time plots of their characteristic masses.

Qualitative identification of a compound was made using the three criteria listed in the protocol: 1) retention time must coincide with known retention times, 2) the three characteristic masses must elute simultaneously, and 3) intensities of the characteristic masses must stand in the known proper proportions.

Quantitation of volatile organics was initially made using peak area counts and concentration calibration curves. Later in the program, response ratios using the 1,4-dichlorobutane internal standard were used in quantifying the concentrations. Base/neutral and acid organic compounds were quantified using deuterated anthracene and response ratios as prescribed in the protocol (1).

Figure 13 is a simplified diagram of the analytical scheme for volatile organic analysis.

Nonvolatile Organics

This method determined the nonvolatile solvent-extractable organic compounds that could be analyzed by gas chromatographic methods. The 129 consent decree priority pollutants contain 81 organic compounds classified as nonvolatile organics.

Nonvolatile organics are divided into three groups: base/neutral fraction, acid fraction (phenols), and pesticides and polychlorinated biphenyls (PCB). A list of compounds that are classified as nonvolatile organics is given in Appendix B.

The analytical procedure is described in Reference 1. Figure 14 depicts the sample processing scheme for the base/neutral and acid fractions. The sample solution, 2×10^{-3}-m^3, was made alkaline (pH greater than 11) with sodium hydroxide, and then

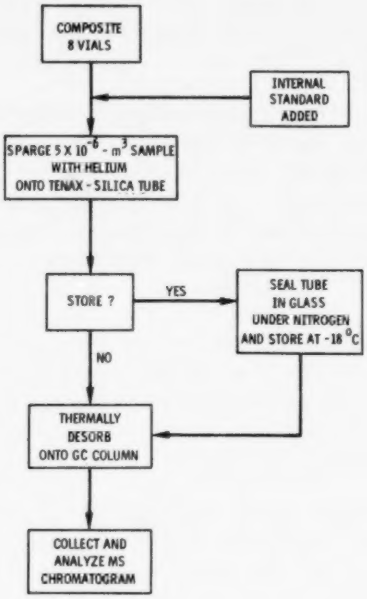

COMPOSITE
8 VIALS
INTERNAL
STANDARD
ADDED
SPARGE 5 X 10^{-6} - m^3 SAMPLE
WITH HELIUM
ONTO TENAX - SILICA TUBE
STORE ?
YES
SEAL TUBE
IN GLASS
UNDER NITROGEN
AND STORE AT -18 °C
NO
THERMALLY
DESORB
ONTO GC COLUMN
COLLECT AND
ANALYZE MS
CHROMATOGRAM

Figure 13. Analytical scheme for volatile organics analysis.

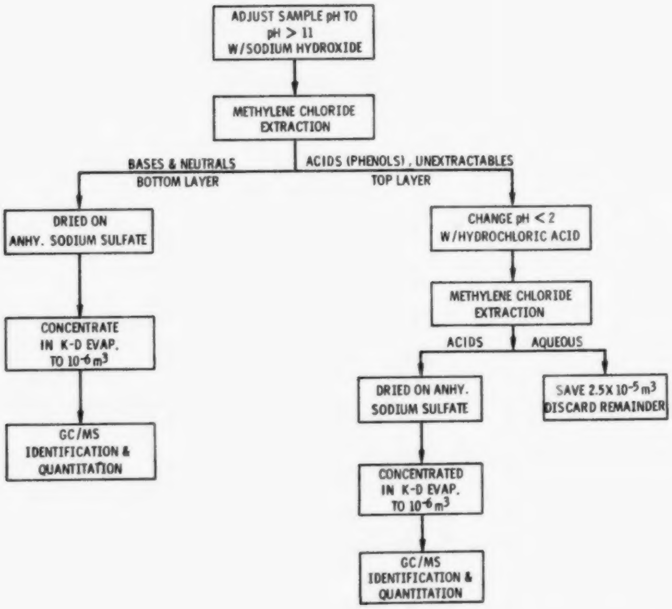

ADJUST SAMPLE pH TO
pH > 11
W/SODIUM HYDROXIDE
METHYLENE CHLORIDE
EXTRACTION
BASES & NEUTRALS
BOTTOM LAYER
ACIDS (PHENOLS), UNEXTRACTABLES
TOP LAYER
DRIED ON
ANHY. SODIUM SULFATE
CHANGE pH < 2
W/HYDROCHLORIC ACID
METHYLENE CHLORIDE
EXTRACTION
CONCENTRATE
IN K-D EVAP.
TO 10^{-6} m^3
ACIDS
AQUEOUS
DRIED ON ANHY.
SODIUM SULFATE
SAVE 2.5 X 10^{-5} m^3
DISCARD REMAINDER
GC/MS
IDENTIFICATION &
QUANTITATION
CONCENTRATED
IN K-D EVAP.
TO 10^{-6} m^3
GC/MS
IDENTIFICATION &
QUANTITATION

Figure 14. Sample processing scheme for nonvolatile organics analysis.

extracted three times with methylene chloride. Textile raw waste and effluent samples formed strong emulsions upon extraction with methylene chloride. The problem was resolved by drawing off small amounts of separated solvent and pouring the extract through the sample in the separatory funnel. Separation was also enhanced by slowly dripping the emulsion onto the wall of a slightly tilted flask. This approach gives better separation by providing a greater surface area for the solvent and water fractions. Some samples required centrifugation at 1,500 rpm for 1 hr to break the emulsion.

Extracts were dried on a column of anhydrous sodium sulfate, concentrated to 10^{-6} m^3 in a Kuderna-Danish (K-D) evaporator with a Snyder column spiked with deuterated anthracene, sealed in septum capped vials, and stored at 4°C until analyzed. Analyses were performed on the GC/MS system using SP 2250 and Tenax GC columns for base/neutral and acid samples, respectively (1).

A separate 0.001-m^3 sample was used for analysis of the pesticides and PCB (Aroclor® fluids). The basic sample processing scheme is shown in Figure 15. These compounds were extracted with a 15% methylene chloride and 85% hexane solvent mixture. The aqueous phase was discarded, and the organic phase was analyzed by GC with an electron capture detector. Where necessary, acetonitrile partitioning and a Florisil® chromatography column were used for further celanup of the sample. In 85% of the samples, additional cleanup was not required.

Confirmation of identify and quantitation were made using two different GC columns: SP-2550 and Dexil 410. Compound verification was made with the MS when the concentration was greater than 0.01 g/m^3. Concentrations of potential pesticides ranged from 0.0001 g/m^3 to 0.01 g/m^3; therefore, MS verification was not possible in this study. Pesticide species identified only by GC below 0.01 g/m^3 were reported only if they met the following two criteria: 1) the retention time window between standards and unknown peaks correlated within ±3 s, and 2) concentrations calculated from both GC columns had to agree within ±20%. Unknown peaks not meeting these criteria were assumed not to be the pesticide species.

Metals

In addition to the volatile and nonvolatile organics, the 129 chemical species include 13 metals, asbestos, and cyanide. Each metal is measured as the total metal. Asbestos was not determined in this study; cyanide was measured by conventional wet chemistry techniques (5).

Eight metals were analyzed by the inductively coupled argon plasma (ICAP) excitation technique: antimony, cadmium, chromium, copper, lead, nickel, silver, and zinc. Five metals which can

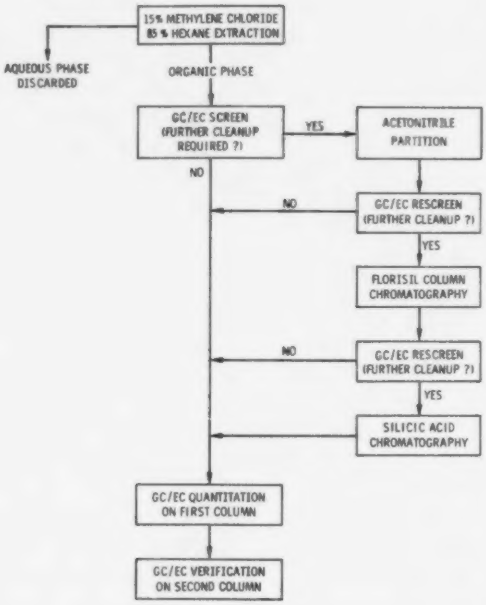

15% METHYLENE CHLORIDE
85 % HEXANE EXTRACTION
AQUEOUS PHASE DISCARDED
ORGANIC PHASE
GC/EC SCREEN (FURTHER CLEANUP REQUIRED ?)
YES
ACETONITRILE PARTITION
NO
NO
GC/EC RESCREEN (FURTHER CLEANUP ?)
YES
FLORISIL COLUMN CHROMATOGRAPHY
NO
GC/EC RESCREEN (FURTHER CLEANUP ?)
YES
SILICIC ACID CHROMATOGRAPHY
GC/EC QUANTITATION ON FIRST COLUMN
GC/EC VERIFICATION ON SECOND COLUMN

Figure 15. Sample processing scheme for pesticide and PCB analysis.

not be quantified by ICAP analysis were measured by conventional atomic absorption techniques: arsenic, beryllium, mercury, selenium, and thallium (4, 5).

ICAP forms an analytical system for simultaneous multielement determinations of trace metals at the sub-ppm level in solutions. The basis of this method is atomic emission. Excitation energy is supplied by coupling a nebulized sample with high temperature argon gas which has been passed through a powerful radio-frequency field. Emitted light is simultaneously monitored at 22 wavelengths corresponding to 22 different elements.

All samples for metals analysis were acidified in the field by adding 5 x 10^{-6} m^3 of redistilled nitric acid to each 10^{-3} m^3 of sample. Nitric acid blanks were also analyzed for metals.

Analytical Results

Raw waste samples were collected with continuous samplers using a peristaltic pump which pulled the sample through Tygon tubing to the sample bottle. Sample blanks were collected by drawing laboratory-prepared organic-free water through the sampler prior to sample collection to determine the presence of base/neutral, acid, and pesticide organic priority pollutants. Results of these analyses are given in Table 10.

TABLE 10. SUMMARY OF CONTINUOUS SAMPLER AND VOLATILE ORGANIC BLANK ANALYSES

Fraction	Compound found	Concentration range, mg/m^3 [a]
Base/neutral	Naphthalene	2
	Dimethylphthalate	16
	Diethylphthalate	0.5 to 10.2
	Bis(2-ethylhexyl)phthalate	1.5 to 46
	Di-n-butylphthalate	1.3 to 1.7
Acids	Phenol	0.6 to 1.1
Volatiles	Toluene	2.6 to 55
	Trans-1,2-dichloroethylene	3.2
	Trichloroethylene	2.4
	Ethylbenzene	8.3
Pesticides and PCB	None	-

[a] 1 mg/m^3 equals 1 μg/l.

To determine if any volatile organic species were absorbed from the air to the samples, EPA recommended that laboratory-prepared organic-free water be carried to the plant site, poured from the container into a sample vial, sealed, and shipped back to the laboratory for analysis (1). These results are included in Table 10.

All secondary effluent samples were collected by grab sampling techniques. Therefore, these samples were not passed through Tygon tubing. Samples were shipped and stored in ice at 4°C until extracted. A special effort was made to initiate methylene chloride extraction as rapidly as possible. The data on which each sample arrived at MRC and the date of its solvent extraction are shown in Table 11. Only 5 out of 64 samples were not extracted within 24-hr of receipt at MRC.

Results of GC/MS analysis of textile raw waste and secondary effluent samples for base/neutral organic compounds are presented in Table 12. GC/MS analyses for the volatile, acid, and pesticide/PCB organic compounds are presented in Table 13.

Of the 114 organic compounds in the priority pollutant list, a total of 45 different compounds were identified in textile wastewaters, 39 in raw waste samples and 34 in secondary effluent samples. The number of compounds found at each plant is summarized in Table 14. On an individual plant basis, the greatest number of organic compounds detected in a raw waste and secondary effluent sample were 14 and 8, respectively, with an average number per plant of 7 in the raw waste and 5 in the secondary effluent.

TABLE 11. TEXTILE SAMPLE EXTRACTION DATES

Plant	Date received at MRC	Date of initial extraction
A	5-6-77	5-6-77
B	4-28-77	4-29-77
C	4-13-77	4-14-77
D	3-3-77	3-3-77
E	3-31-77	3-31-77
F	4-19-77	4-20-77
G-1-2	4-1-77	4-1-77
G-2-2	4-1-77	4-4-77
H	3-10-77	3-10-77
J	3-9-77	3-9-77
K	4-5-77	4-6-77
L	4-12-77	4-12-77
M	3-16-77	3-17-77
N	4-27-77	4-28-77
P	3-2-77	3-2-77
R	3-15-77	3-16-77
S	4-6-77	4-7-77
T	4-26-77	4-27-77
U	5-4-77	5-4-77
V	3-31-77	3-31-77
W	4-14-77	4-14-77
X	4-21-77	4-21-77
Y	3-11-77	3-11-77
Z	3-17-77	3-18-77
C-001	5-20-77	5-20-77
Y-001	5-20-77	5-20-77
JJ	6-23-77	6-23-77
KK	6-24-77	6-27-77
LL	6-27-77	6-29-77
MM	7-5-77	7-8-77
NN	6-30-77	7-1-77
OO	7-5-77	7-6-77
PP	7-5-77	7-8-77

TABLE 12. GC/MS ANALYSES FOR BASE/NEUTRAL ORGANIC COMPOUNDS IN RAW WASTE AND EFFLUENT SAMPLES

Plant/source	Compounds identified and concentrations observed[a]		
	<10 mg/m^3	10 to 100 mg/m^3	>100 mg/m^3
A/raw waste	Naphthalene 0.1 Dimethyl phthalate 3 Diethyl phthalate 1 Bis(2-ethylhexyl) phthalate 0.5	1,4-Dichlorobenzene 11 1,2,4-Trichlorobenzene 90	NPPO[b]
A/effluent	1,2-Dichlorobenzene 1 1,4-Dichlorobenzene 0.05 Bis(2-ethylhexyl) phthalate 6	1,2,4-Trichlorobenzene 46	NPPO
B/raw waste	Diethyl phthalate 3.3 Bis(2-ethylhexyl) phthalate 5.7 Anthracene 0.1	Naphthalene 41	NPPO
B/effluent	N-nitroso-di-n-propylamine 2 Bis(2-ethylhexyl) phthalate 3 Pyrene 0.3	NPPO	NPPO
C/raw waste	1,2-Dichlorobenzene 1.1 Diethyl phthalate 4.1	NPPO	Bis(2-ethylhexyl) phthalate 136
C/effluent	1,2-Dichlorobenzene 0.3 Acenaphthene 0.5 Bis(2-ethylhexyl) phthalate 3.0 Anthracene 4.4	1,2,4-Trichlorobenzene 10.2	NPPO
D/raw waste	Naphthalene 0.3 Bis(2-ethylhexyl) phthalate 8.9	Di-n-butyl phthalate 16.2	NPPO
D/effluent	Diethyl phthalate 1 Bis(2-ethylhexyl) phthalate 5	NPPO	NPPO
E/raw waste	1,4-Dichlorobenzene 2 Napthalene 1 Bis(2-ethylhexyl) phthalate 5	NPPO	NPPO
E/effluent	1,4-Dichlorobenzene 0.2 1,2-Dichlorobenzene 0.2 Dimethyl phthalate 1 Diethyl phthalate 0.5 Pyrene 0.1	Bis(2-ethylhexyl) phthalate 18	NPPO
F/raw waste	1,4-Dichlorobenzene 6.5	1,2-Dichlorobenzene 34.6 Acenaphthene 12.0 Fluorene 14.6 Diethyl phthalate 33.6	1,2,4-Trichlorobenzene 120
F/effluent	1,2,4-Trichlorobenzene 6.3	Bis(2-ethylhexyl) phthalate 23	NPPO
G/raw waste	Fluorene 5	Naphthalene 95 Bis(2-ethylhexyl) phthalate 19	Acenaphthene 273
G/effluent	Acenaphthene 2.0 Hexachlorobenzene 0.8	Diethyl phthalate 11.1 Bis(2-ethylhexyl) phthalate 10.3	NPPO
H/raw waste	1,2-Dichlorobenzene 0.5 Naphthalene 3 Di-n-butyl phthalate 2	Acenaphthene 27 Bis(2-ethylhexyl) phthalate 14	NPPO
H/effluent	NPPO	NPPO	Bis(2-ethylhexyl) phthalate 231
J/raw waste	Diethyl phthalate 6.5	Naphthalene 79.7 Di-n-butyl phthalate 23.2	Bis(2-ethylhexyl) phthalate 162
J/effluent	Di-n-butyl phthalate 3.6 Pyrene 0.1	Bis(2-ethylhexyl) phthalate 35.2	NPPO
K/raw waste	Naphthalene 0.03 Diethyl phthalate 0.2	NPPO	NPPO
K/effluent	Naphthalene 0.5 Bis(2-ethylhexyl) phthalate 8	NPPO	NPPO
L/raw waste	1,4-Dichlorobenzene 1 Bis(2-ethylhexyl) phthalate 3	Acenaphthene 30	Dimethyl phthalate 111
L/effluent	Bis(2-ethylhexyl) phthalate 2	NPPO	NPPO
M/raw waste	NPPO	Naphthalene 92.9	1,2,4-Trichlorobenzene 156 Bis(2-ethylhexyl) phthalate 305
M/effluent	1,2,4-Trichlorobenzene 1.8	Di-n-butyl phthalate 58.4	NPPO
N/raw waste	Diethyl phthalate 5.9	Naphthalene 17 Bis(2-ethylhexyl) phthalate 10.1	1,4-Dichlorobenzene 215 1,2-Dichlorobenzene 287
N/effluent	1,4-Dichlorobenzene 1.5 1,2-Dichlorobenzene 6.0 Diethyl phthalate 9.4	Bis(2-ethylhexyl) phthalate 16.7	NPPO
P/raw waste	Naphthalene 1.9 Diethyl phthalate 1.7 Di-n-butyl phthalate 9.8	Dimethyl phthalate 11.6 Bis(2-ethylhexyl) phthalate 30.2	NPPO
P/effluent	NPPO	Bis(2-ethylhexyl) phthalate 72 N-nitroso-di-n-propylamine 18.9	NPPO
R/raw waste	Di-n-butyl phthalate 7.3	NPPO	Bis(2-ethylhexyl) phthalate 123
R/effluent[c]	Diethyl phthalate 2	Bis(2-ethylhexyl) phthalate 12	NPPO
S/raw waste	NPPO	NPPO	1,2,4-Trichlorobenzene 190 Naphthalene 143 Bis(2-ethylhexyl) phthalate 135
S/effluent	NPPO	Bis(2-ethylhexyl) phthalate 41	1,2,4-Trichlorobenzene 916 Naphthalene 255

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[a] 1 mg/m^3 equals μg/l.

[b] No priority pollutants observed.

[c] Sample inadvertently collected prior to the settling pond.

(continued)

TABLE 12 (continued)

Plant/source	Compounds identified and concentrations observed[a] <10 mg/m³	 10 to 100 mg/m³	 >100 mg/m³
T/raw waste	NPPO[b]	N-nitrosodiphenylamine 11.3	Bis(2-ethylhexyl) phthalate 138
T/effluent	NPPO	Bis(2-ethylhexyl) phthalate 23	NPPO
U/raw waste	1,2-Dichlorobenzene 2.0 Naphthalene 1.5 Diethyl phthalate 6.1	Bis(2-ethylhexyl) phthalate 14.0	NPPO
U/effluent	NPPO	Naphthalene 22	Bis(2-ethylhexyl) phthalate 140
V/raw waste	Acenaphthene 8.7 Bis(2-ethylhexyl) phthalate 5.3 Hexachlorobenzene 2.0	1,2,4-Trichlorobenzene 27.9 Dimethyl phthalate 12.9	NPPO
V/effluent	Bis(2-ethylhexyl) phthalate 9.5	NPPO	NPPO
W/raw waste	Hexachlorobenzene 0.5	Bis(2-ethylhexyl) phthalate 18.1	NPPO
W/effluent	NPPO	Bis(2-ethylhexyl) phthalate 19.0	NPPO
X/raw waste	Naphthalene 1 Bis(2-ethylhexyl) phthalate 1	Acenaphthene 53	NPPO
X/effluent	Diethyl phthalate 3.2 Bis(2-ethylhexyl) phthalate 2.3 Hexachlorobenzene 0.5	NPPO	NPPO
Y/raw waste	1,2-Dichlorobenzene 0.1 Naphthalene 0.4	2,6-Dinitrotoluene 53.5 Bis(2-ethylhexyl) phthalate 87.3	NPPO
Y/effluent	1,2-Dichlorobenzene 0.6 Naphthalene 0.6 Diethyl phthalate 2.6 Di-n-butyl phthalate 6.7 Hexachlorobenzene 0.3	Bis(2-ethylhexyl) phthalate 25.2	NPPO
Z/raw waste	NPPO	1,2,4-Trichlorobenzene 45	Naphthalene 309 Bis(2-ethylhexyl) phthalate 218
Z/effluent	Bis(2-ethylhexyl) phthalate 2	NPPO	NPPO
C-001/raw waste	NPPO	Naphthalene 17.0 Diethyl phthalate 69.0 Bis(2-ethylhexyl) phthalate 23.3	NPPO
Y-001/raw waste	Naphthalene 4 Indeno(1,2,3-cd) pyrene 2 Bis(2-ethylhexyl) phthalate 3	Acenaphthene 13 Diethyl phthalate 15	NPPO
Y-001/effluent	Naphthalene 4.5	Diethyl phthalate 11.7	Bis(2-ethylhexyl) phthalate 134
JJ/raw waste	NPPO	1,2-Dichlorobenzene 11.4	1,2,4-Trichlorobenzene 435
JJ/effluent	NPPO	1,2,4-Trichlorobenzene 32.4	NPPO
KK/raw waste	Diethyl phthalate 2.5 Bis(2-ethylhexyl) phthalate 9.3 Pyrene 0.9	Dimethyl phthalate 11.6	NPPO
KK/effluent	Bis(2-ethylhexyl) phthalate 4.1 Pyrene 0.2	NPPO	NPPO
LL/raw waste	1,2-Dichlorobenzene 0.6	Naphthalene 51.3	1,2,4-Trichlorobenzene 315
LL/effluent	Dimethyl phthalate 0.2 Bis(2-ethylhexyl) phthalate 5.2	NPPO	NPPO
MM/raw waste	NPPO	Bis(2-ethylhexyl) phthalate 15.4	NPPO
MM/effluent	Dimethyl phthalate 0.2 Diethyl phthalate 1.2 Bis(2-ethylhexyl) phthalate 6.9 Bis(2-ethylhexyl) phthalate 2.8 Bis(2-ethylhexyl) phthalate 3.0	(MM-2-1) NPPO (MM-3-1) NPPO (MM-4-1) NPPO	NPPO NPPO NPPO
NN/raw waste	NPPO	Bis(2-ethylhexyl) phthalate 23.1	NPPO
NN/effluent	NPPO	Bis(2-ethylhexyl) phthalate 27.3	NPPO
OO/raw waste	NPPO	Bis(2-ethylhexyl) phthalate 26.0 Di-n-butyl phthalate 61.4	NPPO
OO/effluent	Bis(2-ethylhexyl) phthalate 3.2	NPPO	NPPO
PP/raw waste	NPPO	Butyl benzyl phthalate 72.8 Naphthalene 44.3	NPPO

---

[a] 1 mg/m³ equals 1 μg/l.

[b] No priority pollutant observed.

TABLE 13. GC/MS ANALYSES FOR VOLATILE, ACID, PESTICIDE, AND PCB ORGANIC COMPOUNDS IN RAW WASTE AND EFFLUENT SAMPLES

	Compounds identified and concentrations observed[a]		
Plant/source	Volatiles, mg/m^3	Acids, mg/m^3	Pesticides and PCB, mg/m^3
A/raw waste	NPPO[b]	Phenol 1.2 Pentachlorophenol 71	Heptachlor 6.37
A/effluent	Toluene 8.4	NPPO	Heptachlor 1.55
B/raw waste	Chloroform 3.04 Toluene 3.74	NPPO	NPPO
B/effluent	Trichlorofluoromethane 2.60	NPPO	NPPO
C/raw waste	Trichloroethylene 17.8 Toluene 236 Ethylbenzene 112 1,1,2,2-Tetrachloroethylene 26.4	Phenol 0.5	NPPO
C/effluent	Toluene 2.6 Ethylbenzene 2.0	NPPO	NPPO
D/raw waste	Chloroform 3.3 Toluene 2.3 Ethylbenzene 57.3	Pentachlorophenol 22	NPPO
D/effluent	Toluene 1.67	NPPO	NPPO
E/raw waste	Benzene 5.4 Toluene 61.1 Ethylbenzene 20.7 Chloroform 21.5 *Trans*-1,2-dichloroethylene 1.8 1,1,1-trichloroethane 16.7 Trichloroethylene 2.0 Chlorobenzene 1.0	Phenol 5.7 Pentachlorophenol 30.1	NPPO
E/effluent	Toluene 5.5	NPPO	NPPO
F/raw waste	Trichlorofluoromethane 45 1,1-Dichloroethane 0.59 1,2-Dichloropropane 1.50 1,1,1-Trichloroethane 11.26 *Cis*-1,3-dichloropropene 2.08 Toluene 12.28	Phenol 8.2 Pentachlorophenol 2.4	NPPO
F/effluent	Trichlorofluoromethane 1.73 *Trans*-1,3-dichloropropene 3.90 *Cis*-1,3-dichloropropene 5.61 Toluene 0.85 Ethylbenzene 2.66	2,4-Dimethylphenol 9	NPPO
G/raw waste	Chloroform 5.19	Phenol 0.8	NPPO
G/effluent	Toluene 0.8	Phenol 2	NPPO
H/raw waste	Toluene 25.7 Ethylbenzene 5.7	Phenol 63 2-Nitrophenol 60 *p*-Chloro-*m*-cresol 4.5 4-Nitrophenol 65	NPPO
H/effluent	Toluene 11.9 Trichlorofluoromethane 2,138	NPPO[b]	NPPO
J/raw waste	Toluene 36.1	NPPO	NPPO
J/effluent	Toluene 8.0	NPPO	NPPO
J/effluent	Toluene 8.0 Ethylbenzene 50.8	NPPO	NPPO

---

[a] 1 mg/m^3 equals 1 μg/l.

[b] No priority pollutant observed.

(continued)

TABLE 13 (continued)

	Compounds identified and concentrations observed[a]		
Plant/source	Volatiles, mg/m^3	Acids, mg/m^3	Pesticides and PCB, mg/m^3
K/raw waste	Chloroform 4.8 Toluene 29.3 Ethylbenzene 63.8	2,4,6-Trichlorophenol 0.7 Pentachlorophenol 3.9	NPPO
K/effluent	Chloroform 58.1 Trichloroethylene 4.6 Toluene 24.0 Ethylbenzene 0.7	NPPO	γ-BHC 0.31
L/raw waste	Chloroform 2.5 Toluene 5.2 Ethylbenzene 2.0	NPPO	NPPO
L/effluent	Benzene 0.5	NPPO	NPPO
M/raw waste	NPPO[b]	Phenol 12.4 Pentachlorophenol 6.9	NPPO
M/effluent	Toluene 0.4	NPPO	NPPO
N/raw waste	Trichloroethylene 20.8 Toluene 43.8 Ethylbenzene 1,770	Phenol 11	NPPO
N/effluent	Toluene 16.6 Ethylbenzene 75.0	2,4-Dimethylphenol 8	NPPO
P/raw waste	Chloroform 17.3 Toluene 36.1 Ethylbenzene 1,209 Chlorobenzene 24.8	Phenol 6.6	NPPO
P/effluent	Chloroform 6.9 Toluene 22.4 Ethylbenzene 278	NPPO	NPPO
R/raw waste	Chloroform 33.2 Benzene 31.0 Toluene 281 Ethylbenzene 2,835 Chlorobenzene 296 1,1,2,2-Tetrachloroethylene 15.1	NPPO	NPPO
R/effluent[c]	Toluene 16.8 Ethylbenzene 28.7	Chloro cresol 32 Pentachlorophenol 56	NPPO
S/raw waste	Chloroform 71.1 1,1,2,2-Tetrachloroethylene 38.7 Chlorobenzene 13.6 Toluene 60.7 Ethylbenzene 851.7	NPPO	β-BHC 0.35
S/effluent	Toluene 21.4 Ethylbenzene 109 1,1,2,2-Tetrachloroethylene 0.4	NPPO	NPPO
T/raw waste	Toluene 303 Ethylbenzene 18.4 1,1,2,2-Tetrachloroethylene 6.4	NPPO	NPPO

---

[a] 1 mg/m^3 equals 1 μg/l.

[b] No priority pollutant observed.

[c] Sample inadvertently collected prior to the settling pond.

(continued)

TABLE 13 (continued)

	Compounds identified and concentrations observed[a]		
Plant/source	Volatiles, mg/m^3	Acids, mg/m^3	Pesticides and PCB, mg/m^3
T/effluent	Toluene 33.1 1,1,2,2-Tetrachloroethylene 2.9	NPPO[b]	NPPO
U/raw waste	1,1-Dichloroethane 3.67 1,1,1-Trichloroethane 306	Phenol 0.7 Pentachlorophenol 1.6	NPPO
U/effluent	Chloroform 18.05 Bromodichloromethane 1.54 *Trans*-1,3,-dichloropropene 0.89 Toluene 13.03	NPPO	NPPO
V/raw waste	Toluene 8.4 Ethylbenzene 4.9	NPPO	NPPO
V/effluent	Toluene 1,401	NPPO	NPPO
W/raw waste	Trichloroethylene 13.1 Benzene 19.4 Toluene 62.2 Ethylbenzene 1.1	Phenol 100	NPPO
W/effluent	Toluene 1.7	NPPO	NPPO
X/raw waste	1,1,1-Trichloroethane 8.2 Toluene 63.5 Ethylbenzene 369 1,1,2,2-Tetrachlorethylene 414.2	Phenol 3.8	NPPO
X/effluent	Toluene 39.6 Trichlorofluoromethane 35.0 1,1,2,2-Tetrachloroethylene 40.5	NPPO	NPPO
Y/raw waste	NPPO	Phenol 10.0	NPPO
Y/effluent	Chloroform 4.8 Trichlorofluoromethane 10.1	NPPO	NPPO
Z/raw waste	Toluene 5.5 Ethylbenzene 0.7 1,1,2,2-Tetrachloroethylene 12.0	Phenol 34	NPPO
Z/effluent	Toluene 110.6 Ethylbenzene 3,018 Chlorobenzene 3.5 Trichlorofluoromethane 89.3	NPPO	NPPO
C-001/raw waste	Toluene 5.7 Trichlorofluoromethane 26.8	NPPO	NPPO
Y-001/raw waste	Chloroform 14.3 Chlorobenzene 1.6 Chlorobenzene 1.6 Toluene 11.6 Ethylbenzene 1.9	Phenol 19	NPPO
Y-001 effluent	Toluene 15.1	Phenol 2.9 *p*-Chloro-*m*-cresol 1.6	NPPO
JJ/raw waste	Trichloroethylene 187 1,1,2,2-Tetrachloroethylene 1,126 Ethylbenzene 14	Phenol 41.4	Not analyzed

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[a] 1 mg/m^3 equals 1 μg/l.

[b] No priority pollutant observed.

(continued)

TABLE 13 (continued)

	Compounds identified and concentrations observed[a]		
Plant/source	Volatiles, mg/m^3	Acids, mg/m^3	Pesticides and PCB, mg/m^3
JJ/effluent	Trichloroethylene 84	NPPO[b]	Not analyzed
KK/raw waste	Trichloroethylene 52 Toluene 28 Chlorobenzene 42 Ethylbenzene 26	2-Chlorophenol 131 2,4,6-Trichlorophenol 20.2 Pentachlorophenol 20.4	Not analyzed
KK/effluent	Benzene 64 Chlorobenzene 26	2-Chlorophenol 10 2,4,6-Trichlorophenol 21.1	Not analyzed
LL/raw waste	Chloroform 498 Trichloroethylene 121 1,1,2,2-Tetrachloroethylene 1,108 Ethylbenzene 484	Phenol 16.1	Not analyzed
LL/effluent	NPPO	NPPO	Not analyzed
MM-1/raw waste	NPPO	NPPO	Not analyzed
MM-2/effluent	NPPO	NPPO	Not analyzed
MM-3/effluent	NPPO	NPPO	Not analyzed
MM-4/effluent	Toluene 2	NPPO	Not analyzed
NN/raw waste	NPPO	Phenol 10.1	Not analyzed
NN/effluent	NPPO	NPPO	Not analyzed
OO/raw waste	Chloroform 48 Trichloroethylene 42	Phenol 22.9	Not analyzed
OO/effluent	Chloroform 10 Toluene 3	NPPO	Not analyzed
PP/raw waste	Benzene 200 Toluene 83 Ethylbenzene 42	NPPO	Not analyzed

[a] 1 mg/m^3 equals 1 µg/l.

[b] No priority pollutant observed.

TABLE 14. NUMBER OF PRIORITY ORGANIC POLLUTANTS FOUND IN THE RAW WASTE AND SECONDARY EFFLUENT STREAMS

	Number of organic compounds detected[a]	
Plant	In raw waste	In secondary effluent
A	9	6
B	6	4
C	8	7
D	7	3
E	13	7
F	14	8
G	6	6
H	11	3
J	5	5
K	5	7
L	7	2
M	5	3
N	10	7
P	10	5
R	8	3[b]
S	9	8
T	5	3
U	8	6
V	7	2
W	7	2
X	8	7
Y	5	8
Z	8	5
C-001	5	NA[c]
Y-001	11	6
JJ[d]	6	2
KK[d]	11	6
LL[d]	8	2
MM[d]	1	4
NN[d]	2	1
OO[d]	5	3
PP[d]	5	NA

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[a] In the list of priority pollutants there are 114 organic compounds.

[b] Sample inadvertently collected prior to the setting pond.

[c] Not applicable; these plants discharge their wastewater to a municipal treatment system.

[d] Pesticides were not analyzed at this plant.

The frequency of occurrence of 45 organic species in 64 wastewater samples is given in Table 15. Dominant compounds were bis(2-ethylhexyl)phthalate found in 54 samples, toluene found in 44 samples, and ethylbenzene found in 30 samples.

Results of metal analyses by inductively coupled argon plasma (ICAP) and atomic absorption (AA) analysis are presented in Tables 16 and 17.

Table 16 lists concentrations for the metals included in the 129 consent decree priority pollutants list (Appendix A). Table 17 lists data for the additional elements automatically measured in ICAP analysis. The upper and lower rows of numbers for each plant correspond to metal concentrations in the raw wastewater and secondary effluent, respectively. All metal concentrations are reported as g/m^3 (ppm).

The lower detection limits for routine ICAP and AA analyses, referred to in footnote "a" of Tables 16 and 17, are given in Table 18.

LEVEL 1 CHEMICAL ANALYSIS

Analysis Procedures

The EPA-Process Measurements Branch, IERL/RTP, has developed a phased sampling and analytical strategy for environmental assessment programs (2). Level 1 is the first part of a three-phase approach for performing the assessments. The Level 1 chemical analysis procedure, including modifications to accommodate the special requirements of textile wastewaters, are discussed in this section.

Level 1 chemical analyses were performed on samples from only 15 of the 23 basic textile plants because this task was implemented by EPA after the program began. Eight-hour composited grab samples were collected from the secondary effluent at the 15 plants.

Figure 16 is a schematic diagram for field handling of wastewater samples as recommended by EPA (2). The procedure specifies collection of 0.02 m^2 of composite sample, which is divided into two 0.01 m^3 portions of organic and inorganic chemical analysis. Part of the inorganic composite (0.001 m^3) is set aside for determination of BOD_5 and COD. The remainder is filtered in the field with Gelman Spectro-Grade glass fiber filters (or equivalent). Tared filters are sent to the laboratory for analysis of inorganic elements, leachable anions, and total suspended solids.

Filtrate is extracted in the field with methylene chloride to separate organic from inorganic chemical species. The aqueous portion is divided into three parts for analysis.

TABLE 15. OCCURRENCE OF PRIORITY ORGANIC POLLUTANTS COMBINED FROM RAW WASTE AND SECONDARY EFFLUENT SAMPLES

Priority pollutant	Number of Samples in which pollutant was detected[a]			Observed concentration range,[b,c] mg/m³
	Total	Raw waste samples	Secondary effluent samples	
Bis(2-ethylhexyl)phthalate	54	27	27	0.5 to 300
Toluene	44	22	22	0.4 to 300
Ethylbenzene	30	20	10	0.7 to 3,000
Naphthalene	25	20	5	0.03 to 300
Diethyl phthalate	21	12	9	0.2 to 70
Phenol	21	19	2	0.5 to 100
Chloroform	17	12	5	2 to 500
1,2,4-Trichlorobenzene	14	8	6	2 to 900
1,2-Dichlorobenzene	13	8	5	0.1 to 300
1,1,2,2-Tetrachloroethylene	11	8	3	0.4 to 2,100
Trichloroethylene	10	8	2	2 to 200
Acenaphthene	9	7	2	0.5 to 270
Di-n-butyl phthalate	9	6	3	2 to 60
Pentachlorophenol	8	8	0	2 to 70
Dimethyl phthalate	8	5	3	0.2 to 110
1,4-Dichlorobenzene	8	5	3	0.05 to 200
Chlorobenzene	8	6	2	1 to 300
Trichlorofluoromethane	8	2	6	2 to 2,100
Benzene	6	4	2	0.5 to 200
1,1,1-Trichloroethane	5	5	0	2 to 300
Pyrene	5	1	4	0.1 to 0.9
Hexachlorobenzene	5	2	3	0.3 to 2
2,4,6-Trichlorophenol	3	2	1	0.7 to 20
N-nitroso-di-n-propylamine	3	1	2	2 to 20
N-nitrosodiphenylamine	3	1	2	2 to 20
Heptachlor	2	1	1	2 to 6
Anthracene	2	1	1	0.1 to 4
Fluorene	2	2	0	5 to 15
1,1-Dichloroethane	2	2	0	0.6 to 4
Cis-1,3-dichloropropene	2	1	1	2 to 6
Trans-1,3-dichloropropene	2	0	2	0.9 to 4
2,4-Dimethylphenol	2	0	2	8 to 9
2-Chlorophenol	2	1	1	10 to 130
α-BHC	1	0	1	0.3
β-BHC	1	1	0	0.4
2,6-Dinitrotoluene	1	1	0	50
Indeno(1,2,3-cd)pyrene	1	1	0	2
Butylbenzyl phthalate	1	0	1	70
Trans-1,2-dichloroethylene	1	1	0	2
1,2-Dichloropropane	1	1	0	2
2-Nitrophenol	1	1	0	70
4-Nitrophenol	1	1	0	70
Chloro cresol	1	0	1	30
Bromodichloromethane	1	0	1	2

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[a] Out of a total of 64 samples.

[b] Rounded to one significant figure.

[c] 1 mg/m³ equals 1 μg/l.

TABLE 16. CONSENT DECREE METALS CONCENTRATIONS IN WASTEWATER SAMPLES

Plant code	Metals concentration - raw waste, g/m^3 - secondary effluent, g/m^3												
	Silver	Arsenic	Beryllium	Cadmium	Chromium	Copper	Mercury	Nickel	Lead	Antimony	Selenium	Thallium	Zinc
A	a a	a a	b b	a a	0.19 0.18	0.021 0.027	0.004 a	0.009 0.14	a a	a 0.03	a a	a a	1.3 6.4
B	a a	a a	b b	0.0007 a	0.012 0.004	0.074 0.03	0.0009 0.0006	a a	a a	a a	a a	a a	0.3 0.17
C	a a	a a	a a	0.005 0.006	0.035 0.031	0.008 0.020	a 0.0007	0.15 0.14	0.12 0.12	0.007 0.004	a a	a a	0.074 0.12
D	0.011 a	0.017 0.006	a a	a a	a a	0.031 a	a a	0.03 a	a a	0.003 0.002	a a	a a	0.21 0.21
E	0.007 a	a a	a a	0.006 0.001	0.011 0.004	0.84 0.03	a a	0.04 0.04	0.008 a	0.008 0.0008	a a	a a	7.9 5.1
F	0.10 0.08	a a	b b	0.01 0.01	0.006 0.004	0.59 0.13	a 0.0009	0.10 0.06	0.08 0.0006	0.001 0.0003	a a	a a	0.26 0.57
G	0.0085 a	a a	a a	a a	0.004 0.003	0.063 0.028	a a	0.028 0.013	0.006 a	0.052 0.011	a a	a a	0.45 0.26
H	0.041 a	a a	a a	a a	0.004 a	0.022 a	a a	0.014 a	a a	0.004 0.006	a a	a a	3.9 0.96
J	0.06 a	a a	a a	a a	0.048 0.025	2.4 0.1	a a	0.097 0.09	0.029 a	0.0007 a	a a	a a	2.1 0.8
K	0.13 a	0.006 a	a a	0.004 a	0.019 0.004	0.026 0.015	a a	0.1 a	0.03 a	0.003 0.0008	a a	a a	0.15 0.11
L	a a	a a	a a	a a	0.003 0.03	0.30 0.096	a a	0.054 0.035	0.036 a	0.005 0.003	a a	a a	1.0 0.72
M	a a	a a	a a	a a	a a	0.009 0.005	a a	a a	a a	0.0008 0.004	a a	a a	1.2 0.41
N	a a	a a	b b	0.046 a	0.88 1.8	0.020 0.008	0.0004 a	a 0.03	a a	0.0002 0.002	a a	a a	7.5 38.4
P	0.03 0.008	a a	a a	a a	0.003 a	a a	a a	0.10 0.04	0.013 a	a a	a a	a a	0.20 0.14
R[c]	a a	a a	a a	a a	0.067 0.14	0.51 0.29	a a	a a	a a	a a	a a	a a	0.24 0.21
S	a a	0.005 a	a a	a a	0.0007 a	0.04 0.06	a a	a	a a	0.057 0.074	a a	a a	0.12 0.084

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[a] Metals concentration below instrument detection limit - see Table 18 for detection limit.

[b] Analysis not performed.

[c] Secondary effluent sample inadvertently collected prior to the settling pond.

TABLE 16 (continued)

Plant code	Metals concentration - raw waste, g/m^3 - secondary effluent, g/m^3												
	Silver	Arsenic	Beryllium	Cadmium	Chromium	Copper	Mercury	Nickel	Lead	Antimony	Selenium	Thallium	Zinc
T	a a	a a	b b	a a	a a	0.12 0.06	0.0007 a	0.05 0.004	0.025 a		a a	a a	0.29 0.08
U	a a	a a	b b	a a	0.027 0.014	0.040 0.023	0.0004 a	0.008	a a	0.007 0.001	a a	a a	0.26 0.19
V	a a	a a	a a	0.005 a	0.004 0.003	0.23 0.17	a a	a a	a a	a 0.004	a a	a a	0.46 0.34
W	0.065 0.095	a 0.004	a a	0.009 0.013	0.012 0.003	0.023 0.002	a 0.0005	0.054 0.060	0.018 0.057	a a	a a	a a	0.19 0.09
X	0.017 0.033	a a	b b	0.005 0.007	0.024 0.039	0.084 0.11	a 0.0009	0.11 0.072	0.032 0.026	0.0003 0.0009	a a	a a	0.034 0.078
Y	a a	a a	a a	a a	0.026 0.001	0.096 0.11	a a	0.012 a	a a	0.016 0.003	a a	a a	0.24 0.091
Z	a a	a a	a a	a a	a a	0.097 0.050	a a	0.011 a	a a	0.011 0.012	a a	a a	0.11 0.37
Y-001	0.068 0.057	a a	b b	0.006 0.007	0.65 0.29	0.041 a	b b	0.20 0.16	0.16 0.16	a a	b b	b b	0.13 0.10
C-001	0.033	a	b	0.003	0.024	a	b	0.099	0.073	b	b	b	0.056
JJ	0.047 0.049	0.20 0.16	b b	0.005 0.005	0.16 0.08	0.032 0.031	b b	0.10 0.12	0.084 0.065	b b	b b	b b	0.13 0.32
KK	0.022 0.044	0.12 a	b b	0.002 0.004	0.016 0.013	0.086 0.037	b b	0.077 0.11	0.049 0.044	b b	b b	b b	1.08 0.39
LL	0.058 0.056	0.10 0.07	b b	0.004 0.002	0.011 0.020	0.038 0.092	b b	0.13 0.15	0.060 0.048	b b	b b	b b	0.067 0.068
MM-1 -2 -3 -4	0.016 0.025 0.028 0.032	0.055 0.003 0.007 0.006	b b b b	0.002 0.002 a 0.002	0.111 0.058 0.11 0.13	0.036 0.059 0.028 0.042	b b b b	0.044 0.072 0.067 0.081	0.011 0.037 0.031 0.035	b b b b	b b b b	b b b b	0.24 0.19 0.28 0.37
NN	0.042 0.033	a a	b b	0.002 0.004	0.023 0.17	0.047 0.046	b b	0.098 0.079	0.033 0.025	b b	b b	b b	0.084 0.13
OO	0.046 0.050	a a	b b	0.004 0.005	0.011 0.012	0.039 0.037	b b	0.11 0.12	0.043 0.084	b b	b b	b b	0.12 2.3
PP	0.048	a	b	0.005	0.010	0.041	b	0.12	0.078	b	b	b	0.073

[a] Metals concentration below instrument detection limit - see Table 18 for detection limit.

[b] Analysis not performed.

[c] Secondary effluent sample inadvertently collected prior to the settling pond.

TABLE 17. OTHER METALS GIVEN BY ICAP ANALYSIS

Plant code	Metals concentration - raw waste, g/m^3 - secondary effluent, g/m^3														
	Aluminum	Barium	Boron	Calcium	Cobalt	Iron	Magnesium	Manganese	Molybdenum	Sodium	Silicon	Tin	Phosphorus	Titanium	Vanadium
A	0.24 0.23	0.064 0.030	0.15 0.18	18 28	0.0042 0.013	0.72 2.1	3.6 4.0	0.05 0.07	a a	>100 >100	3.3 1.4	0.05 0.02	1.2 0.50	0.012 0.008	0.060 0.59
B	0.16 0.024	0.037 0.008	0.07 0.043	20 12	0.0056 0.0052	0.32 0.27	9.5 4.67	0.093 0.059	a a	>100 >100	4.4 2.3	0.018 0.041	12 6.5	0.0039 a	0.058 0.030
C	0.30 0.19	0.083 0.073	0.39 0.15	5.0 4.5	0.0081 0.0056	1.0 0.22	3.9 0.73	0.029 0.017	0.019 0.024	>100 >100	16 15	0.07 0.12	4.0 4.1	0.020 0.012	0.29 0.40
D	0.38 0.15	a a	1.4 0.90	3.6 6.6	0.001 0.001	0.39 0.46	3.1 4.6	0.03 0.07	a a	>100 >100	6.8 7.3	0.02 0.02	1.6 1.0	0.008 0.0001	0.03 0.03
E	0.028 0.038	0.015 0.012	2.36 1.06	5.4 39	a a	0.12 0.62	2.5 3.1	0.027 0.10	a 0.019	>100 54	11 8.6	0.03 0.01	1.9 1.4	0.018 0.012	0.019 0.021
F	0.11 0.06	0.03 0.03	0.84 0.68	4.1 4.2	0.0041 0.012	0.33 0.28	3.1 3.2	0.007 0.009	0.025 0.023	>100 >100	10 13	0.07 0.04	24 9.5	0.0028 0.0039	0.014 0.013
G	0.52 0.23	0.008 0.015	0.69 0.22	4.2 3.1	0.0008 0.011	0.17 0.39	0.52 0.49	0.05 0.038	0.0028 a	97 55	2.4 3.2	0.03 0.02	6.4 6.1	0.011 a	0.020 0.022
H	0.093 0.055	a a	a a	1.3 6.2	0.0054 a	0.16 0.40	2.6 3.2	0.013 0.009	0.011 a	>100 >100	17 15	0.019 0.055	0.99 0.20	0.009 a	0.035 0.032
J	0.95 0.01	0.12 0.024	a a	4.9 5.2	0.0084 0.0009	0.70 0.52	5.8 6.9	0.05 0.05	0.0043 a	>100 >100	18 17	0.03 0.05	3.3 0.6	1.5 0.06	0.13 0.11
K	0.28 a	0.028 0.018	13 1.1	5.8 3.6	0.009 0.007	0.67 0.088	4.2 3.7	0.030 0.011	0.020 0.0006	>100 >100	23 15	0.05 0.04	1.9 0.93	0.0035 0.0104	0.012 0.035
L	0.095 0.064	0.008 0.019	6.1 8.9	3.4 6.3	0.0012 0.0012	0.18 0.46	1.3 2.4	0.022 0.021	a a	>100 >100	6.0 6.9	0.019 0.007	2.2 1.6	0.010 0.0013	0.012 0.032
M	0.33 0.009	0.013 a	0.91 0.53	9.7 8.8	0.0077 0.0045	0.18 0.06	6.7 6.4	0.034 0.14	a a	>100 >100	15 14	4.0 3.5	3.99 3.46	0.0092 a	0.042 0.037
N	0.29 1.2	0.018 0.16	0.041 0.058	10 26	0.007 0.030	1.4 4.7	1.68 4.2	0.46 1.1	a 0.008	>100 >100	11 6.9	0.012 a	0.43 5.2	0.010 0.001	0.013 0.037
P	0.62 0.14	a a	0.20 0.52	1.6 9.5	0.007 0.003	0.72 0.12	0.48 1.8	0.042 0.02	0.002 a	76 >100	5.7 4.8	0.06 0.004	5.7 2.2	0.006 a	0.02 0.02
R[c]	0.28 0.07	0.003 a	0.21 0.21	7.5 5.1	0.028 0.026	0.30 0.13	3.6 3.0	0.018 0.013	a a	>100 >100	13 11	0.003 a	3.9 0.66	0.071 a	0.021 0.016
S	0.068 0.91	0.005 0.028	1.6 1.5	2.8 7.3	0.001 0.001	0.12 0.31	1.8 2.8	0.021 0.03	a a	>100 >100	14 15	0.007 0.018	1.6 5.0	0.0005 0.0005	0.008 0.024

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[a] Metals concentration below instrument detection limit - see Table 18 for detection limits.

[b] Secondary effluent sample only.

[c] Secondary effluent samples inadvertently collected prior to settling pond.

TABLE 17 (continued)

Plant code	Aluminum	Barium	Boron	Calcium	Cobalt	Iron	Magnesium	Manganese	Molybdenum	Sodium	Silicon	Tin	Phosphorus	Titanium	Vanadium
	Metals concentration - waste, g/m^3 - secondary effluent, g/m^3														
T	0.20	0.013	0.73	12	0.008	0.14	4.2	0.18	a	>100	15	0.042	12	0.0009	0.045
	0.075	0.008	0.34	9.9	0.003	0.43	3.5	0.17	a	>100	6.6	0.028	17	0.0021	0.037
U	0.90	0.01	0.032	5.8	0.005	0.68	2.5	0.076	a	>100	3.7	0.028	3.5	0.006	0.023
	0.17	0.006	0.28	8.0	a	0.30	2.5	0.016	a	>100	3.0	a	3.7	0.003	0.019
V	0.20	0.014	3.4	3.2	a	0.37	1.4	0.063	a	94	4.5	0.013	0.75	0.003	0.019
	0.16	0.013	0.49	3.6	0.004	0.47	1.7	0.072	a	>100	4.5	0.005	0.78	a	0.013
W	6.0	0.33	0.44	93	0.14	5.7	14	0.89	a	65	20	0.030	5.1	0.035	0.14
	0.77	0.15	0.14	36	0.005	1.7	5.0	0.38	0.021	51	10	0.030	0.15	0.012	0.036
X	0.14	0.028	0.28	8.3	0.034	0.22	1.4	0.020	0.008	82	7.9	0.006	4.6	0.011	0.022
	0.94	0.005	0.31	9.3	0.024	0.35	1.9	0.010	0.012	>100	8.4	0.002	5.4	0.001	0.029
Y	0.076	0.13	1.4	2.1	0.026	0.14	3.2	0.044	a	>100	9.8	0.001	16	0.001	0.019
	0.018	0.029	2.3	8.9	0.004	0.17	5.8	0.046	a	>100	8.2	0.002	15	a	0.037
Z	0.023	a	0.12	2.5	0.009	0.16	0.65	0.014	a	>100	8.8	a	1.1	0.002	0.089
	a	a	0.36	2.1	0.006	0.075	1.2	0.010	a	>100	6.2	a	0.5	a	0.086
Y-001	0.57	0.015	0.045	68	0.32	1.2	3.1	0.12	0.031	65	7.8	0.068	11.7	0.020	0.039
	6.3	0.011	0.68	61	0.27	1.1	3.0	0.08	0.042	59	7.7	0.058	6.8	0.008	0.088
C-001	0.29	0.070	0.11	22	0.019	2.9	8.1	0.45	0.011	>100	12	0.040	2.7	0.010	0.072
JJ	2.7	0.048	0.23	7.3	0.012	2.3	2.2	0.14	0.026	88	9.8	0.057	3.5	0.096	0.044
	1.8	0.042	0.24	16.5	0.010	1.5	4.0	0.19	0.024	>100	7.8	0.042	2.3	0.056	0.049
KK	0.38	0.060	1.0	12.4	0.004	0.42	14.4	0.054	0.010	>100	31.9	0.077	6.3	0.042	0.091
	0.26	0.051	0.85	11.8	0.009	0.46	11.6	0.036	0.015	>100	27.8	0.064	6.4	0.017	0.077
LL	0.22	0.008	0.043	4.5	0.004	0.16	2.0	0.040	0.015	>100	5.7	0.033	18.8	0.014	0.032
	0.17	0.004	0.091	3.7	0.004	0.10	2.0	0.021	0.016	>100	7.1	0.031	28.8	0.008	0.086
MM-1	0.44	0.010	1.1	5.7	0.004	0.39	3.7	0.030	0.001	>100	7.7	0.028	1.9	0.006	0.036
-2	3.7	0.005	1.0	5.1	0.009	0.30	3.5	0.025	0.010	>100	5.9	0.021	0.78	0.011	0.065
-3	0.48	0.020	1.1	4.3	0.005	0.25	2.9	0.017	0.004	>100	7.6	0.009	1.9	0.009	0.034
-4	0.50	0.011	1.1	8.9	0.006	0.40	3.8	0.032	0.004	>100	7.7	0.003	2.1	0.008	0.039
NN	1.0	0.002	0.043	0.85	0.010	0.28	0.26	0.12	0.005	>100	2.3	0.052	48.8	0.006	0.019
	1.3	0.001	0.800	5.7	0.008	0.75	0.55	0.008	0.006	>100	1.6	0.051	46.8	0.007	0.025
OO	0.24	0.018	1.1	3.3	0.004	0.37	1.3	0.020	0.001	52	5.2	0.053	4.6	0.004	0.020
	0.15	0.016	1.1	3.5	0.008	0.10	0.88	0.019	0.012	66	6.3	0.036	0.66	0.006	0.020
PP[b]	0.052	0.004	0.017	0.021	0.007	0.009	0.0004	0.002	0.001	0.196	0.059	0.002	0.08	0.0001	0.006

[a] Metals concentration below instrument detection limit - see Table 18 for detection limits.

[b] Secondary effluent sample only.

[c] Secondary effluent samples inadvertently collected prior to settling pond.

TABLE 18. LOWER DETECTION LIMIT OF METALS ANALYSIS SYSTEMS

Metal	Detection limit, g/m^3	Metal	Detection limit, g/m^3
Metals analyzed by ICAP[a]			
Aluminum	0.050	Manganese	0.005
Arsenic	0.005	Molybdenum	0.0006
Barium	0.0002	Nickel	0.01
Boron	0.0001	Phosphorus	0.01
Cadmium	0.0005	Sodium	0.050
Calcium	0.0002	Silicon	0.003
Chromium	0.0002	Silver	0.0050
Cobalt	0.0005	Tin	0.001
Copper	0.0002	Titanium	0.001
Iron	0.005	Vanadium	0.002
Lead	0.001	Zinc	0.025
Magnesium	0.001		
Metals analyzed by atomic absorption			
Antimony	0.0005	Selenium	0.005
Beryllium	0.0001	Thallium	0.005
Mercury	0.0005		

[a] 1 g/m^3 equals 1 mg/l.

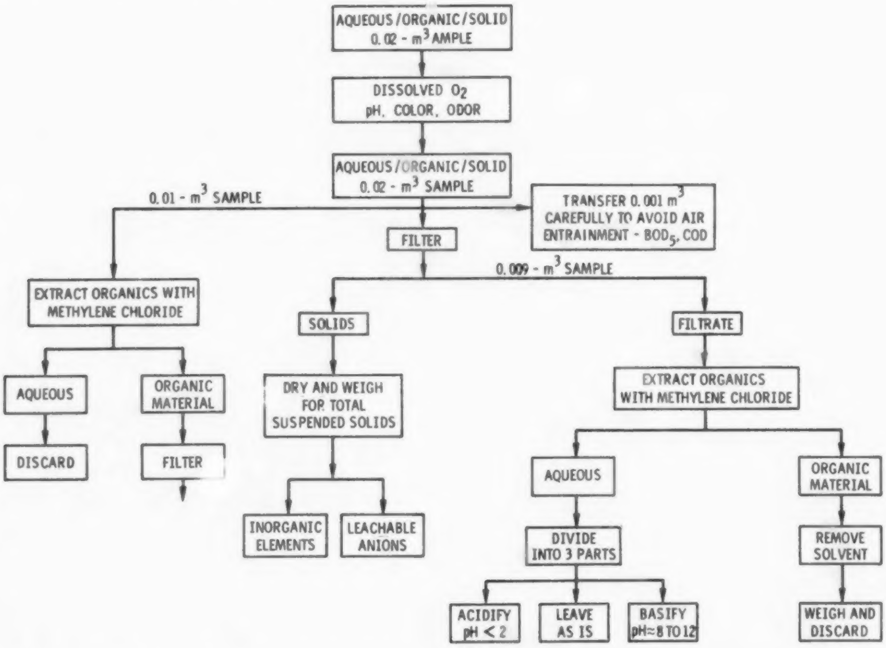
AQUEOUS/ORGANIC/SOLID 0.02 - m^3 AMPLE
DISSOLVED O_2 pH, COLOR, ODOR
AQUEOUS/ORGANIC/SOLID 0.02 - m^3 SAMPLE
0.01 - m^3 SAMPLE
TRANSFER 0.001 m^3 CAREFULLY TO AVOID AIR ENTRAINMENT - BOD_5, COD
FILTER
0.009 - m^3 SAMPLE
EXTRACT ORGANICS WITH METHYLENE CHLORIDE
SOLIDS
FILTRATE
AQUEOUS
ORGANIC MATERIAL
DRY AND WEIGH FOR TOTAL SUSPENDED SOLIDS
EXTRACT ORGANICS WITH METHYLENE CHLORIDE
DISCARD
FILTER
INORGANIC ELEMENTS
LEACHABLE ANIONS
AQUEOUS
ORGANIC MATERIAL
DIVIDE INTO 3 PARTS
REMOVE SOLVENT
ACIDIFY pH < 2
LEAVE AS IS
BASIFY pH≈8 TO 12
WEIGH AND DISCARD

Figure 16. EPA-recommended field handling scheme for liquid/slurry samples (1).

The 0.01 m^3 portion for organic analysis is extracted in the field with methylene chloride. The aqueous portion is discarded; the methylene chloride extract is filtered and sent to the laboratory for organic chemical analysis.

Because textile wastewaters from stable emulsions with methylene chloride, the field handling procedure was modified, with the approval of the EPA-Process Measurements Branch, IERL/RTP. It was not feasible to conduct methylene chloride extractions in the field because previous experience has shown that it requires from 1 hr to 3 hr of concerted effort to break these emulsions. Centrifugation was necessary occasionally. The modified field handling scheme for textile wastewater is shown in Figure 17. The fundamental difference between the two schemes is that the field analysis of some of the common wastewater chemical species is performed on unextracted water samples. Cyanide samples were not extracted before analysis.

The filter obtained from the field was dried and the concentration of total suspended solids determined. Leachate analyses were not performed because each textile plant sampled was meeting its EPA effluent standard for TSS at the time samples were collected. The filter paper was ashed by means of low temperature plasma, digested and analyzed for metals by spark source mass spectrometry (SSMS) and conventional atomic absorption (AA). Level 1 chemical analysis protocoal requires SSMS for metals analysis and AA for those metals not accurately detected by SSMS.

SSMS can be used for analysis of nonvolatile compounds, such as inorganic solids and trace elements. The spark produces ions from the sample by high voltage breakdown across two electrodes. One electrode usually consists of or contains the sample material. In the spark ion source, the spark is sustained between ions from the rf spark source are accelerated through a potential field and focused with dual collimating slits. The ion beam passes into the mass spectrometer where the ions are separated. Photographic plates are used to record the emissions spectra for various mass fractions that correspond to specific elements or compounds.

A detailed flow diagram for Level 1 organic analysis is shown in Figure 18 (2). First, 0.01 m^3 of the sample is extracted with methylene chloride. The aqueous phase is saved and the organic phase separated for analysis.

The modification to the Level 1 organic analysis scheme, as recommended by the EPA, was employed in addition to the basic Level 1 chemical analysis procedure.

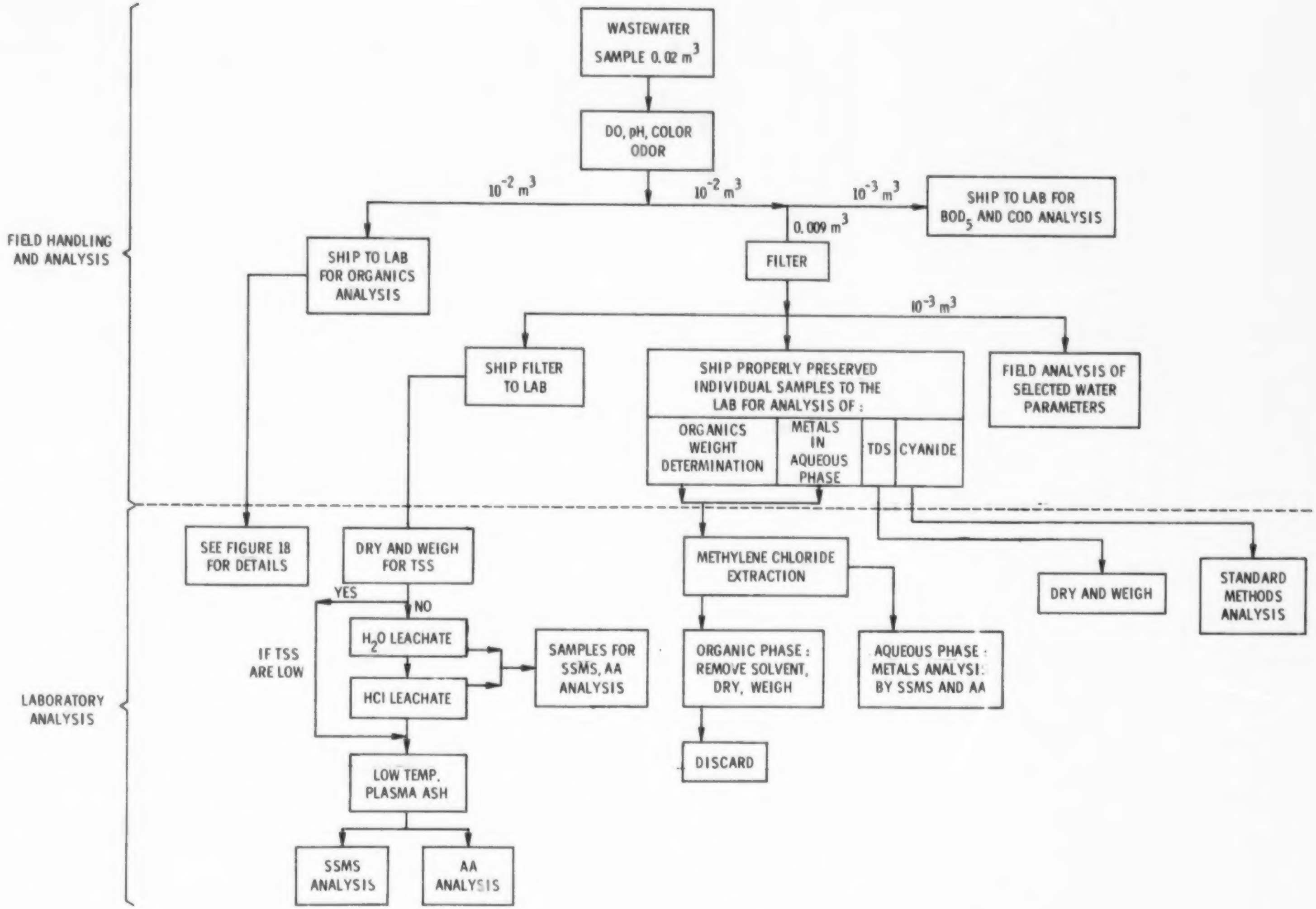
FIELD HANDLING AND ANALYSIS
LABORATORY ANALYSIS
WASTEWATER SAMPLE 0.02 m^3
DO, pH, COLOR ODOR
10^{-2} m^3
10^{-2} m^3
10^{-3} m^3
SHIP TO LAB FOR BOD$_5$ AND COD ANALYSIS
0.009 m^3
SHIP TO LAB FOR ORGANICS ANALYSIS
FILTER
10^{-3} m^3
SHIP FILTER TO LAB
SHIP PROPERLY PRESERVED INDIVIDUAL SAMPLES TO THE LAB FOR ANALYSIS OF :
ORGANICS WEIGHT DETERMINATION
METALS IN AQUEOUS PHASE
TDS
CYANIDE
FIELD ANALYSIS OF SELECTED WATER PARAMETERS
SEE FIGURE 18 FOR DETAILS
DRY AND WEIGH FOR TSS
YES
NO
IF TSS ARE LOW
H$_2$O LEACHATE
HCl LEACHATE
SAMPLES FOR SSMS, AA ANALYSIS
LOW TEMP. PLASMA ASH
SSMS ANALYSIS
AA ANALYSIS
METHYLENE CHLORIDE EXTRACTION
ORGANIC PHASE : REMOVE SOLVENT, DRY, WEIGH
DISCARD
AQUEOUS PHASE : METALS ANALYSIS BY SSMS AND AA
DRY AND WEIGH
STANDARD METHODS ANALYSIS

Figure 17. MRC-modified Level 1 field sampling and analysis scheme.

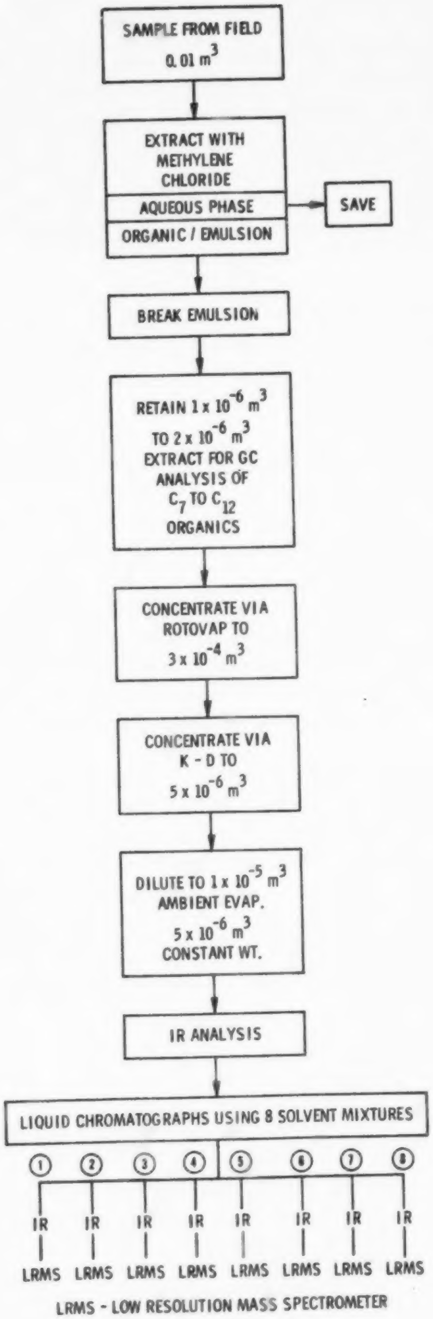
SAMPLE FROM FIELD
0.01 m3
EXTRACT WITH METHYLENE CHLORIDE
AQUEOUS PHASE
ORGANIC / EMULSION
SAVE
BREAK EMULSION
RETAIN 1 x 10-6 m3 TO 2 x 10-6 m3 EXTRACT FOR GC ANALYSIS OF C7 TO C12 ORGANICS
CONCENTRATE VIA ROTOVAP TO 3 x 10-4 m3
CONCENTRATE VIA K - D TO 5 x 10-6 m3
DILUTE TO 1 x 10-5 m3 AMBIENT EVAP. 5 x 10-6 m3 CONSTANT WT.
IR ANALYSIS
LIQUID CHROMATOGRAPHS USING 8 SOLVENT MIXTURES
① ② ③ ④ ⑤ ⑥ ⑦ ⑧
IR IR IR IR IR IR IR IR
LRMS LRMS LRMS LRMS LRMS LRMS LRMS LRMS
LRMS - LOW RESOLUTION MASS SPECTROMETER

Figure 18. Level 1 organic analysis scheme (2).

Funds to perform the additional methods development required for the modification were supplied under another EPA contract.[a]

The principal feature of this modification is that the organic extract is not evaporated to dryness prior to liquid chromatography (LC) and/or low resolution mass spectrometry (LRMS). Two new sample handling steps are required:

- A quantitative assay procedure using GC/MS to complement gravimetric analysis and give quantitative data on C_7-C_{12} hydrocarbons.
- A solvent exchange step to transfer the sample from methylene chloride extract to nonpolar solvent and allow lower boiling material (less than C_7) to pass through the chromatograph and subsequent steps.

The new flow diagram incorporating these changes is shown in Figure 19.

Results of Inorganic Analyses

The Level 1 chemical analysis scheme is divided into inorganic and organic analysis. Inorganic analysis includes: BOD_5, COD, TSS, TDS, metals analysis by spark source mass spectrometer (SSMS) of filtered solids and the filtrate, field analysis of selected species, and total nonvolatile organic concentration.

Table 19 shows the concentrations of the following parameters in 15 plant effluents: BOD_5, COD, TSS, TDS, and total organic concentration. Data from the field analysis of effluent samples are found in Table 20. Metals concentrations of the suspended solids collected on the filter paper are listed in Table 21. Results of SSMS analyses of the filtrate are given in Table 22. Concentrations are reported as g/m^3 (ppm) of textile effluent sample. All elements for which values are not entered have concentrations below the detection limit.

Results of Organic Analyses

As illustrated in Figures 17 and 19, there are four points within the Level 1 chemical analysis scheme where organic analysis takes place. At each plant one portion of the sample was filtered and a part of this volume was extracted with methylene chloride. The aqueous phase was used for metals analysis by SSMS, and the organic phase was dried and weighed to determine the concentration of total methylene chloride extractable organics. Results of these analyses are shown in Table 23.

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[a] Contract No. 68-02-1411, Task 19, "Analysis Support of Textile Environmental Assessments."

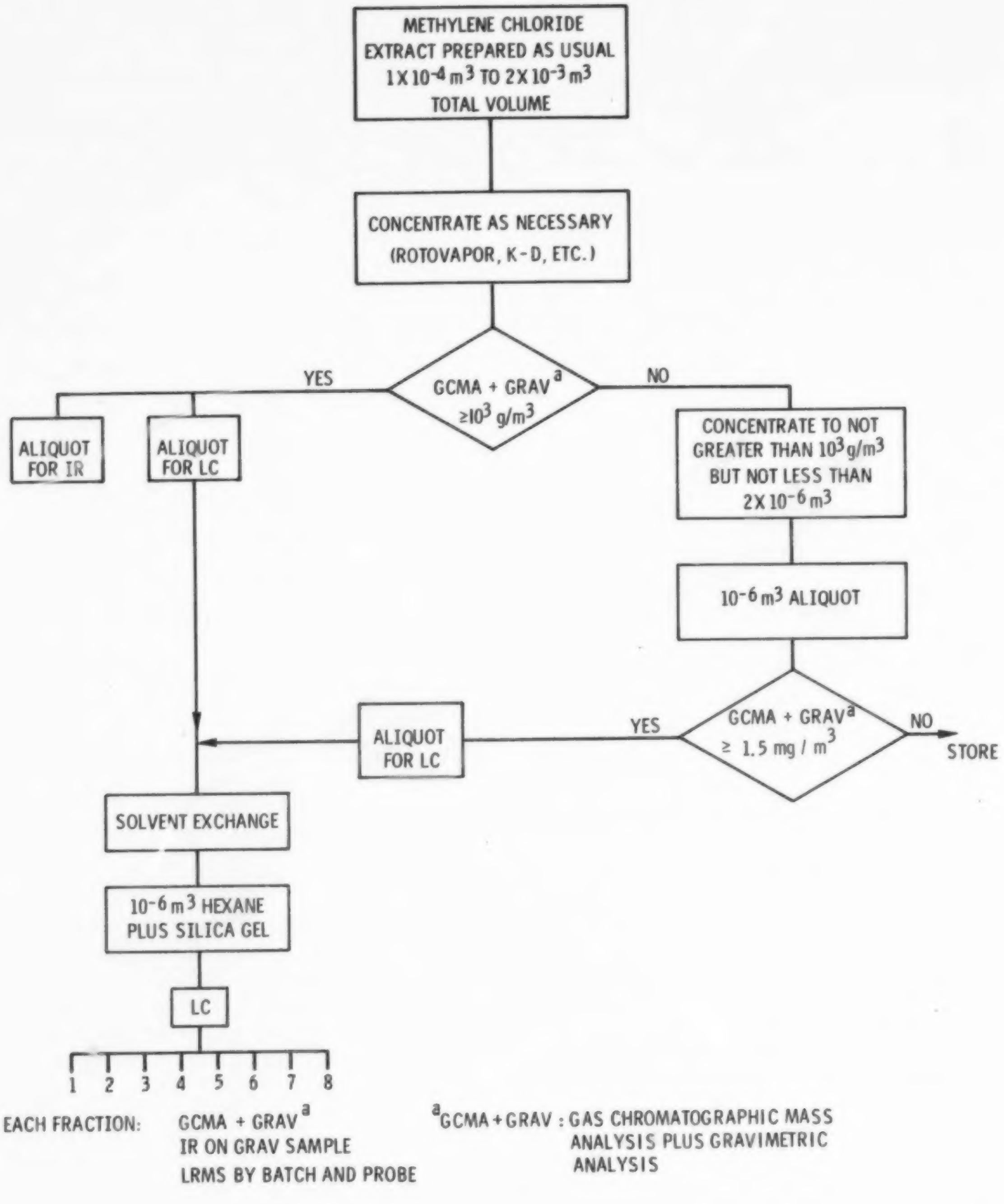
METHYLENE CHLORIDE EXTRACT PREPARED AS USUAL $1 \times 10^{-4}\ m^3$ TO $2 \times 10^{-3}\ m^3$ TOTAL VOLUME
CONCENTRATE AS NECESSARY (ROTOVAPOR, K-D, ETC.)
YES
GCMA + GRAV[a] $\geq 10^3\ g/m^3$
NO
ALIQUOT FOR IR
ALIQUOT FOR LC
CONCENTRATE TO NOT GREATER THAN $10^3\ g/m^3$ BUT NOT LESS THAN $2 \times 10^{-6}\ m^3$
$10^{-6}\ m^3$ ALIQUOT
ALIQUOT FOR LC
YES
GCMA + GRAV[a] $\geq 1.5\ mg/m^3$
NO
STORE
SOLVENT EXCHANGE
$10^{-6}\ m^3$ HEXANE PLUS SILICA GEL
LC
1 2 3 4 5 6 7 8
EACH FRACTION: GCMA + GRAV[a]
IR ON GRAV SAMPLE
LRMS BY BATCH AND PROBE
[a]GCMA + GRAV : GAS CHROMATOGRAPHIC MASS ANALYSIS PLUS GRAVIMETRIC ANALYSIS

Figure 19. EPA, IERL/RTP modification to Level 1 organics analysis procedure.

TABLE 19. SELECTED PARAMETERS OF TEXTILE EFFLUENT SAMPLES FROM THE INORGANIC SEGMENT OF LEVEL 1 CHEMICAL ANALYSIS PROTOCOL

Plant code	5-day biochemical oxygen demand, g/m^3 [a]	Chemical oxygen demand g/m^3	Total suspended solids, g/m^3	Total dissolved solids, g/m^3	Total organic concentration, g/m^3
A	168	1652	234	1,725	63.7
B	b	99	7	1,681	3.18
C	25	396	24	2,924	28.2
E	<5	78	10	13,120	3.60
F	69	276	10	2,006	16.0
G	42	502	5	276	27.2
K	<5	131	14	1,256	2.73
L	13	234	42	725	18.3
N	36	286	13	1,352	9.24
S	59	1035	349	692	5.40
T	32	414	44	660	17.8
U	24	748	111	1,331	14.6
V	<5	128	b	b	b
W	84	837	217	1,648	15.0
X	15	258	1.3	437	13.5

[a] 1 g/m^3 equals 1 mg/l.
[b] Analysis not performed.

Portions (1 to 2 x 10^{-6} m^3) of each sample were then analyzed by gas chromatography (GC) to determine the concentration of C_7 to C_{12} hydrocarbons. The Following GC columsn were used: a) 1.8 m x 3.2 mm stainless steel column packed with 10% VC, W98 on 80 to 100S, and b) 1.8 m x 3.2 mm stainless steel column packed with 10% SP-2100. Each column was held at 50°C for 4 min, then programmed at 16°C/min to 250°C and held at 250°C for 4 min.

Results of these analyses indicated that in 13 of the 14 samples the C_7 to C_{12} hydrocarbon concentrations were below the threshold detection limit of 1.0 g/m^3 (ppm). The secondary effluent sample from plant X contained a total concentration of about 3.0 g/m^3 of C_7 to C_{12} hydrocarbons.

TABLE 20. FIELD ANALYSIS OF SELECTED SECONDARY WASTEWATER PARAMETERS ON ILTERED, UNEXTRACTED SAMPLES AS PER LEVEL 1 ANALYSIS PROTOCOL (2).

			Water parameters, g/m^3[a]										
Plant code	Color, APHA	Specific conductivity[b]	Nitrate	Nitrite	Hydrogen sulfide	Sulfate	Methyl orange acidity	Dissolved oxygen	Ammonia	*o*-phosphate	Total alkalinity	Chromium	pH
A	2,000	1,500	1.9	0.06	4	8.5	0	5.5	12.8	1.0	100	0.18	7.3
B	90	1,200	0.002	<0.005	0.20	368	0	7	2.5	7.3	5.5	0.004	7.5
C	1,920	2,400	23.3	4.64	5	40	0	6	3.4	1.08	8.3	0.031	10
E	0	310	79.2	0.016	0.1	12	0	8.5	3.4	2.0	35	0.004	7.5
F	80	1,900	0	0.043	0.1	10	0	5	1.54	0.56	2.4	0.004	7.4
G	500	155	1.32	0.076	<2	56	0	8	72.5	4	30	0.003	7.5
K	270	875	4.4	0.056	2	>1	0	5	3.1	2.5	710	0.004	7.2
L	370	555	13.5	0.864	3	460	0	4	0.5	0.88	30	0.03	5.8
N	90	990	5.5	0.003	0.1	640	20	9	12.8	11.2	0	1.8	7.0
S	240	640	4.4	0.033	0.3	150	0	7	72.5	72	130	0	7.8
T	350	460	0.8	0.04	6	100	0	8	13.6	6.4	300	0	7.4
U	2,480	770	0.8	<0.005	3.5	0	0	9	5.44	2.96	120	0.014	7.3
V	500	360	0.88	0.264	0.5	57	0	9	2.5	1.7	0.4	0.003	7.1
W	1,900	1,250	12.3	0.145	0.1	0	0	5	0.38	0.075	950	0.003	8.1
X	>10	285	0.033	0.44	0.01	1	0	7.2	0.05	0	140	0.039	7.2

[a] 1 g/m^3 equals 1 mg/l.

[b] Units μmhos at 25°C.

TABLE 21. LEVEL 1 SPARK SOURCE MASS SPECTROMETER METALS ANALYSIS OF THE SUSPENDED SOLIDS COLLECTED ON THE FILTER PAPER DURING FIELD FILTRATION

Plant A		Concentration: mg/m^3		Detection limit: 0.01 mg/m^3			
Uranium	<0.04	Terbium	<0.03	Ruthenium		Vanadium	318
Thorium	<0.04	Gadolinium	<0.04	Molybdenum	17	Titanium	9.5
Bismuth	3.0	Europium	<0.04	Niobium	0.03	Scandium	0.07
Lead	42	Samarium	<0.07	Zirconium	1.9	Calcium	1,220
Thallium	<0.04	Neodymium	<0.09	Yttrium	0.08	Potassium	265
Mercury	–[a]	Praseodymium	<0.05	Strontium	6.0	Chlorine	15
Gold		Cerium	1.3	Rubidium	1.0	Sulphur	1,987
Platinum		Lanthanum	0.91	Bromine	3.0	Phosphorus	1,590
Iridium		Barium	15	Selenium	0.25	Silicon	450
Osmium		Cesium		Arsenic	3.4	Aluminum	874
Rhenium	–[b]	Iodine	0.33	Germanium	0.03	Magnesium	702
Tungsten	<0.08	Tellurium		Gallium	0.13	Sodium	4,636
Tantalum	<0.03	Antimony	1.6	Zinc	300	Fluorine	28
Hafnium	<0.11	Tin	13	Copper	172	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	26	Nitrogen	–[a]
Ytterbium	<0.04	Cadmium	2.5	Cobalt	2.0	Carbon	–[a]
Thullium	<0.03	Silver	1.1	Iron	582	Boron	–[a]
Erbium	<0.03	Palladium		Manganese	11	Beryllium	<0.04
Holmium		Rhodium		Chromium	56	Lithium	1.3
Dysprosium	<0.01						

Plant B		Concentration: mg/m^3		Detection limit: 0.01 g/m^3			
Uranium		Terbium		Ruthenium		Vanadium	0.02
Thorium		Gadolinium		Molybdenum		Titanium	0.3
Bismuth	0.05	Europium		Niobium	0.04	Scandium	
Lead	0.2	Samarium		Zirconium		Calcium	30
Thallium		Neodymium	0.1	Yttrium		Potassium	11
Mercury	–[a]	Praseodymium		Strontium	0.1	Chlorine	0.3
Gold		Cerium	0.02	Rubidium	0.08	Sulphur	24
Platinum		Lanthanum	0.01	Bromine	0.02	Phosphorus	120
Iridium		Barium	1.8	Selenium		Silicon	340
Osmium		Cesium		Arsenic		Aluminum	25
Rhenium	–[b]	Iodine		Germanium		Magnesium	130
Tungsten		Tellurium		Gallium		Sodium	240
Tantalum	0.003[c]	Antimony		Zinc	1.8	Fluorine	6.8
Hafnium	<0.02	Tin	0.01	Copper	0.3	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	0.07	Nitrogen	–[a]
Ytterbium		Cadmium		Cobalt		Carbon	–[a]
Thullium		Silver		Iron	13	Boron	–[a]
Erbium		Palladium		Manganese	0.3	Beryllium	
Holmium		Rhodium		Chromium	0.5	Lithium	0.04
Dysprosium							

Plant C		Concentration: mg/m^3		Detection limit: 0.05 mg/m^3			
Uranium		Terbium		Ruthenium		Vanadium	1.1
Thorium		Gadolinium	0.07	Molybdenum	0.1	Titanium	0.8
Bismuth	0.7	Europium		Niobium		Scandium	0.1
Lead	0.6	Samarium	0.07	Zirconium	0.2	Calcium	83
Thallium	0.07	Neodymium		Yttrium	0.03	Potassium	330
Mercury	–[a]	Praseodymium		Strontium	1.6	Chlorine	43
Gold		Cerium	0.1	Rubidium	2.8	Sulphur	110
Platinum		Lanthanum	0.07	Bromine	1.1	Phosphorus	700
Iridium		Barium	57	Selenium		Silicon	1,100
Osmium		Cesium	0.03	Arsenic	0.07	Aluminum	530
Rhenium	–[b]	Iodine	0.03	Germanium		Magnesium	160
Tungsten	0.07	Tellurium		Gallium		Sodium	2,800
Tantalum	0.01[c]	Antimony	0.1	Zinc	29	Fluorine	33
Hafnium	0.1	Tin	0.2	Copper	4	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	0.9	Nitrogen	–[a]
Ytterbium	0.07	Cadmium	0.07	Cobalt	0.07	Carbon	–[a]
Thullium		Silver		Iron	20	Boron	–[a]
Erbium	0.07	Palladium		Manganese	0.9	Beryllium	
Holmium		Rhodium		Chromium	0.8	Lithium	0.3
Dysprosium							

(continued)

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[a] Not reported. [b] Internal standard. [c] Instrument source.

TABLE 21 (continued)

Plant E		Concentration: mg/m^3		Detection limit: <0.02 mg/m^3			
Uranium		Terbium		Ruthenium		Vanadium	
Thorium		Gadolinium		Molybdenum	0.04	Titanium	0.05
Bismuth	0.07	Europium		Niobium		Scandium	<0.04
Lead	0.09	Samarium		Zirconium	0.02	Calcium	3.9
Thallium		Neodymium		Yttrium		Potassium	110
Mercury	–[a]	Praseodymium	0.02	Strontium	0.32	Chlorine	18
Gold		Cerium	0.02	Rubidium	0.49	Sulphur	0.74
Platinum		Lanthanum	0.02	Bromine	2.8	Phosphorus	0.04
Iridium		Barium	0.09	Selenium		Silicon	3.5
Osmium		Cesium		Arsenic	<0.07	Aluminum	18
Rhenium	–[b]	Iodine	0.16	Germanium		Magnesium	5.8
Tungsten	0.09	Tellurium		Gallium		Sodium	972
Tantalum		Antimony	0.02	Zinc	0.12	Fluorine	11
Hafnium	<0.05	Tin	0.04	Copper	0.04	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	0.05	Nitrogen	–[a]
Ytterbium	<0.04	Cadmium	0.02	Cobalt		Carbon	–[a]
Thullium		Silver		Iron	0.94	Boron	–[a]
Erbium		Palladium		Manganese	0.05	Beryllium	
Holmium		Rhodium		Chromium	0.07	Lithium	0.02
Dysprosium							

Plant F		Concentration: mg/m^3		Detection limit: 0.05 mg/m^3			
Uranium		Terbium		Ruthenium		Vanadium	0.1
Thorium		Gadolinium		Molybdenum	0.1	Titanium	1.4
Bismuth		Europium		Niobium		Scandium	<0.1
Lead	4.0	Samarium		Zirconium	0.4	Calcium	120
Thallium		Neodymium		Yttrium		Potassium	87
Mercury	–[a]	Praseodymium		Strontium	0.6	Chlorine	34
Gold		Cerium	0.1	Rubidium	0.1	Sulphur	106
Platinum		Lanthanum	0.05	Bromine	1.6	Phosphorus	290
Iridium		Barium	4.3	Selenium		Silicon	820
Osmium		Cesium		Arsenic	0.5	Aluminum	42
Rhenium	–[b]	Iodine		Germanium		Magnesium	30
Tungsten		Tellurium		Gallium		Sodium	1,100
Tantalum	0.002[c]	Antimony	0.4	Zinc	39	Fluorine	82
Hafnium	<0.1	Tin	1.3	Copper	29	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	1.2	Nitrogen	–[a]
Ytterbium		Cadmium	0.2	Cobalt	0.1	Carbon	–[a]
Thullium		Silver		Iron	67	Boron	–[a]
Erbium		Palladium		Manganese	1.1	Beryllium	
Holmium		Rhodium		Chromium	2.0	Lithium	–[a]
Dysprosium							

Plant K		Concentration: mg/m^3		Detection limit: 0.01 mg/m^3			
Uranium		Terbium		Ruthenium		Vanadium	
Thorium		Gadolinium		Molybdenum	0.06	Titanium	0.09
Bismuth		Europium		Niobium		Scandium	<0.03
Lead	0.51	Samarium		Zirconium	0.06	Calcium	100
Thallium	0.01	Neodymium		Yttrium		Potassium	23
Mercury	–[a]	Praseodymium	0.01	Strontium	0.06	Chlorine	1.2
Gold		Cerium	0.03	Rubidium	0.3	Sulphur	18
Platinum		Lanthanum	0.04	Bromine	0.03	Phosphorus	9.7
Iridium		Barium	0.84	Selenium		Silicon	23
Osmium		Cesium		Arsenic	<0.04	Aluminum	45
Rhenium	–[b]	Iodine	0.03	Germanium		Magnesium	7.2
Tungsten	0.06	Tellurium		Gallium		Sodium	308
Tantalum		Antimony	0.01	Zinc	0.75	Fluorine	1.4
Hafnium	<0.03	Tin	0.04	Copper	0.97	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	0.32	Nitrogen	–[a]
Ytterbium		Cadmium		Cobalt		Carbon	–[a]
Thullium		Silver		Iron	3.2	Boron	–[a]
Erbium		Palladium		Manganese	0.1	Beryllium	
Holmium		Rhodium		Chromium	0.81	Lithium	0.03
Dysprosium							

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[a] Not reported. [b] Internal standard. [c] Instrument source.

(continued)

TABLE 21 (continued)

Plant L		Concentration: mg/m^3		Detection limit: 0.05 mg/m^3			
Uranium		Terbium		Ruthenium		Vanadium	1.0
Thorium		Gadolinium		Molybdenum	0.1	Titanium	3.2
Bismuth	0.05	Europium		Niobium		Scandium	<0.1
Lead	9	Samarium	0.3	Zirconium	0.3	Calcium	1,200
Thallium		Neodymium	0.05	Yttrium		Potassium	95
Mercury	–[a]	Praseodymium	0.05	Strontium	1.0	Chlorine	37
Gold		Cerium	0.2	Rubidium	0.3	Sulphur	360
Platinum		Lanthanum	0.1	Bromine	0.4	Phosphorus	1,200
Iridium		Barium	23	Selenium		Silicon	4,600
Osmium		Cesium		Arsenic		Aluminum	440
Rhenium	–[b]	Iodine		Germanium		Magnesium	650
Tungsten		Tellurium		Gallium		Sodium	5,000
Tantalum	0.001[c]	Antimony	0.2	Zinc	1,900	Fluorine	33
Hafnium	<0.1	Tim	0.2	Copper	31	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	3.6	Nitrogen	–[a]
Ytterbium		Cadmium	0.05	Cobalt	0.05	Carbon	–[a]
Thullium		Silver		Iron	380	Boron	–[a]
Erbium	Palladium	Palladium		Manganese	2.2	Beryllium	
Holmium		Rhodium		Chromium	13	Lithium	0.3
Dysprosium							

Plant N		Concentration: mg/m^3		Detection limit: 0.04 mg/m^3			
Uranium		Terbium		Ruthenium		Vanadium	0.4
Thorium		Gadolinium	0.08	Molybdenum	0.2	Titanium	5.6
Bismuth	0.04	Europium	<0.04	Niobium		Scandium	<0.2
Lead	4.0	Samarium	0.1	Zirconium	0.6	Calcium	280
Thallium		Neodymium	0.1	Yttrium	0.4	Potassium	44
Mercury	–[a]	Praseodymium	0.1	Strontium	0.8	Chlorine	3.8
Gold		Cerium	0.5	Rubidium	0.08	Sulphur	1,240
Platinum		Lanthanum	0.1	Bromine	0.1	Phosphorus	2,000
Iridium		Barium	14	Selenium	0.04	Silicon	2,000
Osmium		Cesium		Arsenic	0.8	Aluminum	200
Rhenium	–[b]	Iodine	0.4	Germanium		Magnesium	110
Tungsten	0.04	Tellurium		Gallium	0.04	Sodium	520
Tantalum	0.003[c]	Antimony	0.2	Zinc	150	Fluorine	76
Hafnium	<0.08	Tin	0.3	Copper	12	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	2.9	Nitrogen	–[a]
Ytterbium	<0.08	Cadmium	0.08	Cobalt	1.7	Carbon	–[a]
Thullium		Silver		Iron	600	Boron	–[a]
Erbium		Palladium		Manganese	12	Beryllium	
Holmium		Rhodium		Chromium	680	Lithium	0.2
Dysprosium							

Plant S		Concentration: mg/m^3		Detection limit: 0.01 mg/m^3			
Uranium	0.08	Terbium		Ruthenium		Vanadium	6.1
Thorium	0.21	Gadolinium	0.04	Molybdenum	6.5	Titanium	40
Bismuth	2.4	Duropium	0.05	Niobium	0.1	Scandium	0.14
Lead	7.1	Samarium	0.09	Zirconium	1.6	Calcium	4,353
Thallium	<0.02	Neodymium	1.0	Yttrium	0.33	Potassium	859
Mercury	–[a]	Praseodymium	0.36	Strontium	6.8	Chlorine	129
Gold		Cerium	3.4	Rubidium	11	Sulphur	1,882
Platinum		Lanthanum	4.0	Bromine	14	Phosphorus	4,824
Iridium		Barium	49	Selenium	<0.22	Silicon	1,106
Osmium		Cesium	0.99	Arsenic	7.0	Aluminum	2,000
Rhenium	–[b]	Iodine	0.38	Germanium	0.16	Magnesium	1,035
Tungsten	<0.11	Tellurium		Gallium	0.56	Sodium	694
Tantalum	0.55[c]	Antimony	188	Zinc	13	Fluorine	56
Hafnium	<0.13	Tin	31	Copper	306	Oxygen	–[a]
Lutecium	<0.02	Indium	–[b]	Nickel	16	Nitrogen	–[a]
Ytterbium	<0.07	Cadmium	1.1	Cobalt	0.44	Carbon	–[a]
Thullium	<0.02	Silver	0.25	Iron	2,588	Boron	–[a]
Erbium	<0.04	Palladium		Manganese	56	Beryllium	<0.05
Holmium	0.01	Rhodium		Chromium	26	Lithium	0.58
Dysprosium	0.01						

(continued)

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[a] Not reported. [b] Internal standard. [c] Instrument source.

TABLE 21 (continued)

Plant T Concentration: mg/m^3 Detection limit: 0.01 mg/m^3

Uranium		Terbium		Ruthenium		Vanadium	0.4
Thorium		Gadolinium		Molybdenum	0.2	Titanium	7.3
Bismuth		Europium		Niobium		Scandium	
Lead	53	Samarium		Zirconium	0.9	Calcium	1,500
Thallium		Neodymium		Yttrium	0.2	Potassium	330
Mercury	–[a]	Praseodymium		Strontium	1.6	Chlorine	11
Gold		Cerium	0.4	Rubidium	1.1	Sulphur	140
Platinum		Lanthanum		Bromine	1.8	Phosphorus	1,300
Iridium		Barium	150	Selenium		Silicon	1,040
Osmium		Cesium		Arsenic		Aluminum	550
Rhenium	–[b]	Iodine		Germanium		Magnesium	530
Tungsten		Tellurium		Gallium		Sodium	2,700
Tantalum		Antimony	0.7	Zinc	98	Fluorine	890
Hafnium		Tin	1.1	Copper	29	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	1.6	Nitrogen	–[a]
Ytterbium		Cadmium		Cobalt	0.2	Carbon	–[a]
Thullium		Silver		Iron	320	Boron	–[a]
Erbium		Palladium		Manganese	4.9	Beryllium	
Holmium		Rhodium		Chromium	5.1	Lithium	0.7
Dysprosium							

Plant U Concentration: mg/m^3 Detection limit: <0.01 mg/m^3

Uranium	<0.02	Terbium		Ruthenium		Vanadium	0.49
Thorium	<0.04	Gadolinium		Molybdenum	5.2	Titanium	18
Bismuth	<0.05	Europium	0.02	Niobium		Scandium	0.1
Lead	7.4	Samarium	0.02	Zirconium	2.2	Calcium	756
Thallium	<0.02	Neodymium	0.02	Yttrium	0.16	Potassium	402
Mercury	–[a]	Praseodymium	0.01	Strontium	6.4	Chlorine	12
Gold		Cerium	0.62	Rubidium	3.3	Sulphur	2,680
Platinum		Lanthanum	0.38	Bromine	5.5	Phosphorus	597
Iridium		Barium	12	Selenium	0.09	Silicon	378
Osmium		Cesium	0.01	Arsenic	<0.13	Aluminum	1,220
Rhenium	–[b]	Iodine	0.28	Germanium		Magnesium	116
Tungsten	<0.07	Tellurium		Gallium	0.18	Sodium	1,950
Tantalum	<0.23[c]	Antimony	1.7	Zinc	60	Fluorine	96
Hafnium	<0.09	Tin	4.3	Copper	29	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	11	Nitrogen	–[a]
Ytterbium	<0.06	Cadmium	0.44	Cobalt	0.20	Carbon	–[a]
Thullium		Silver	0.23	Iron	560	Boron	–[a]
Erbium	<0.04	Palladium		Manganese	12	Beryllium	
Holmium		Rhodium		Chromium	23	Lithium	0.40
Dysprosium							

Plant W Concentration: mg/m^3 Detection limit: 0.05 mg/m^3

Uranium		Terbium	0.1	Ruthenium		Vanadium	15
Thorium	0.5	Gadolinium	0.5	Molybdenum	0.5	Titanium	160
Bismuth	<0.2	Europium	0.2	Niobium	0.2	Scandium	0.4
Lead	14	Samarium	<0.9	Zirconium	1.9	Calcium	15,000
Thallium	<0.1	Neodymium	1.5	Yttrium	1.1	Potassium	1,500
Mercury	–[a]	Praseodymium	1.3	Strontium	19	Chlorine	290
Gold		Cerium	8.0	Rubidium	3.1	Sulphur	330
Platinum		Lanthanum	2.3	Bromine	2.5	Phorphorus	1,700
Iridium		Barium	230	Selenium		Silicon	2,600
Osmium		Cesium	0.1	Arsenic	13	Aluminum	6,700
Rhenium	–[b]	Iodine	0.07	Germanium		Magnesium	3,700
Tungsten	<0.2	Tellurium		Gallium	2.3	Sodium	3,300
Tantalum	0.006[c]	Antimony	0.4	Zinc	66	Fluorine	390
Hafnium	<0.3	Tin	1.3	Copper	23	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	17	Nitrogen	–[a]
Ytterbium	<0.2	Cadmium	0.3	Cobalt	8.0	Carbon	–[a]
Thullium		Silver	0.07	Iron	4,500	Boron	–[a]
Erbium	<0.1	Palladium		Manganese	370	Beryllium	0.2
Holmium		Rhodium		Chromium	10	Lithium	13
Dysprosium							

(continued)

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[a] Not reported. [b] Internal standard. [c] Instrument source.

TABLE 21 (continued)

Plant X Concentration: mg/m^3 Detection limit: 0.03 mg/m^3

Uranium		Terbium		Ruthenium		Vanadium	0.4
Thorium		Gadolinium	<0.06	Molybdenum	0.1	Titanium	4.7
Bismuth	<0.09	Europium		Niobium	0.06	Scandium	0.06
Lead	5.0	Samarium	<0.06	Zirconium	0.4	Calcium	1,200
Thallium	<0.06	Neodymium	0.06	Yttrium	0.08	Potassium	53
Mercury	–[a]	Praseodymium	0.06	Strontium	1.3	Chlorine	17
Gold		Cerium	0.2	Rubidium	0.4	Sulphur	110
Platinum		Lanthanum	0.09	Bromine	17	Phosphorus	2,400
Iridium		Barium	22	Selenium		Silicon	880
Osmium		Cesium		Arsenic	<0.06	Aluminum	640
Rhenium	–[b]	Iodine	0.09	Germanium		Magnesium	
Tungsten	0.06	Tellurium		Gallium	0.06	Sodium	2,200
Tantalum	0.08[c]	Antimony	2.4	Zinc	410	Fluorine	38
Hafnium	<0.1	Tin	0.44	Copper	27	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	7	Nitrogen	–[a]
Ytterbium	<0.09	Cadmium	0.06	Cobalt	2.7	Carbon	–[a]
Thullium		Silver		Iron	1,300	Boron	–[a]
Erbium	<0.06	Palladium		Manganese	2.5	Beryllium	
Holmium		Rhodium		Chromium	12	Lithium	0.15
Dysprosium							

[a] Not reported. [b] Internal standard. [c] Instrument source.

TABLE 22. LEVEL 1 SPARK SOURCE MASS SPECTROMETERS METALS ANALYSIS OF FILTERED SECONDARD EFFLUENT (detection limit of 0.001 g/m³)

Plant A, g/m³							
Uranium	<0.003	Terbium		Ruthenium		Vanadium	2.5
Thorium	<0.003	Gadolinium	<0.003	Molybdenum	0.043	Titanium	0.087
Bismuth	<0.006	Europium	<0.002	Niobium		Scandium	<0.006
Lead	0.38	Samarium	<0.002	Zirconium	0.031	Calcium	170
Thallium	<0.004	Neodymium	<0.004	Yttrium	0.003	Potassium	8.8
Mercury	–[a]	Praseodymium	0.009	Strontium	0.70	Chlorine	11
Gold		Cerium	0.002	Rubidium	0.19	Sulphur	130
Platinum		Lanthanum	0.004	Bromine	0.47	Phosphorus	6
Iridium		Barium	0.51	Selenium	0.003	Silicon	18
Osmium		Cesium	0.003	Arsenic	<0.015	Aluminum	6.4
Rhenium	–[b]	Iodine	0.011	Garmanium		Magnesium	10
Tungsten	<0.008	Tellurium		Gallium		Sodium	180
Tantalum	0.008[c]	Antimony	0.095	Zinc	13	Fluorine	1.1
Hafnium	<0.010	Tin	0.10	Copper	0.14	Oxygen	–[a]
Lutecium	<0.002	Indium	–[b]	Nickel	1.0	Nitrogen	–[a]
Ytterbium	<0.007	Cadmium	0.005	Cobalt	0.021	Carbon	–[a]
Thullium	<0.002	Silver	0.001	Iron	6.1	Boron	–[a]
Erbium	<0.007	Palladium		Manganese	0.48	Beryllium	<0.003
Holmium		Rhodium		Chromium	1.4	Lithium	0.12
Dysprosium							
Plant B, g/m³							
Uranium	<0.003	Terbium		Ruthenium		Vanadium	0.005
Thorium	<0.003	Gadolinium	<0.003	Molybdenum	≤0.007	Titanium	0.079
Bismuth	<0.005	Europium	<0.002	Niobium		Scandium	<0.003
Lead	0.17	Samarium	<0.002	Zirconium	0.004	Calcium	21
Thallium	<0.002	Neodymium	<0.003	Yttrium	0.002	Potassium	79
Mercury	–[a]	Praseodymium	≤0.002	Strontium	0.22	Chlorine	2.1
Gold		Cerium	≤0.003	Rubidium	0.25	Sulphur	300
Platinum		Lanthanum	<0.002	Bromine	0.26	Phosphorus	45
Iridium		Barium	0.20	Selenium	0.006	Silicon	8.0
Osmium		Cesium	0.001	Arsenic	0.28	Aluminum	0.96
Rhenium	–[b]	Iodine	0.004	Germanium		Magnesium	14
Tungsten	<0.007	Tellurium		Gallium		Sodium	190
Tantalum	0.10[c]	Antimony	0.019	Zinc	1.2	Fluorine	3.7
Hafnium	<0.009	Tin	0.017	Copper	0.16	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	0.027	Nitrogen	–[a]
Ytterbium	<0.006	Cadmium	0.006	Cobalt	0.008	Carbon	–[a]
Thullium	<0.002	Silver	0.001	Iron	2.4	Boron	–[a]
Erbium	<0.003	Palladium		Manganese	0.41	Beryllium	<0.003
Holmium		Rhodium		Chromium	0.24	Lithium	0.032
Dysprosium							
Plant C, g/m³							
Uranium	<0.005	Terbium	<0.002	Ruthenium		Vanadium	4.4
Thorium	<0.005	Gadolinium	<0.004	Molybdenum	0.011	Titanium	0.71
Bismuth	<0.010	Europium	<0.003	Niobium	0.004	Scandium	<0.017
Lead	<0.25	Samarium	<0.006	Zirconium	0.009	Calcium	41
Thallium	<0.007	Neodymium	<0.005	Yttrium	0.001	Potassium	7.7
Mercury	–[a]	Praseodymium	0.004	Strontium	1.1	Chlorine	2.3
Gold		Cerium	<0.003	Rubidium	0.38	Sulphur	340
Platinum		Lanthanum	<0.004	Bromine	13	Phosphorus	20
Iridium		Barium	1.1	Selenium	0.027	Silicon	27
Osmium		Cesium	0.001	Arsenic	<0.025	Aluminum	0.99
Rhenium	–[b]	Iodine	0.024	Germanium	0.004	Magnesium	1.8
Tungsten	<0.013	Tellurium	<0.002	Gallium		Sodium	370
Tantalum	<0.045[c]	Antimony	0.62	Zinc	1.2	Fluorine	12
Hafnium	<0.017	Tin	0.082	Copper	0.63	Oxygen	–[a]
Lutecium	<0.003	Indium	–[b]	Nickel	0.45	Nitrogen	–[a]
Ytterbium	<0.011	Cadmium	0.030	Cobalt	0.018	Carbon	–[a]
Thullium	<0.003	Silver	10	Iron	2.1	Boron	–[a]
Erbium	<0.007	Palladium		Manganese	0.18	Beryllium	<0.005
Holmium	<0.002	Rhodium		Chromium	0.053	Lithium	0.044
Dysprosium	<0.003						

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[a] Not reported. [b] Internal standard. [c] Instrument source.

(continued)

TABLE 22 (continued)

Plant E, g/m³

Uranium		Terbium		Ruthenium		Vanadium	0.018
Thorium		Gadolinium		Molybdenum	0.004	Titanium	0.13
Bismuth	<0.002	Europium		Niobium		Scandium	
Lead	0.063	Samarium	<0.002	Zirconium	0.009	Calcium	36
Thallium	<0.002	Neodymium	0.007	Yttrium		Potassium	29
Mercury	–[a]	Praseodymium	0.004	Strontium	0.094	Chlorine	1.3
Gold		Cerium	0.022	Rubidium	0.071	Sulphur	290
Platinum		Lanthanum	0.019	Bromine	0.92	Phosphorus	9.2
Iridium		Barium	0.29	Selenium	0.002	Silicon	2.9
Osmium	–[b]	Cesium		Arsenic	0.035	Aluminum	0.39
Rhenium		Iodine	0.011	Germanium		Magnesium	2.1
Tungsten	<0.003	Tellurium		Gallium		Sodium	70
Tantalum	0.001[c]	Antimony	0.13	Zinc	0.76	Fluorine	13
Hafnium	<0.003	Tin	0.006	Copper	0.10	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	0.038	Nitrogen	–[a]
Ytterbium	<0.002	Cadmium	0.004	Cobalt	0.001	Carbon	–[a]
Thullium		Silver	0.001	Iron	0.86	Boron	–[a]
Erbium	<0.002	Palladium		Manganese	0.035	Beryllium	
Holmium		Rhodium		Chromium	0.043	Lithium	0.014
Dysprosium							

Plant F, g/m³

Uranium	<0.003	Terbium		Ruthenium		Vanadium	0.022
Thorium	<0.004	Gadolinium	<0.003	Molybdenum	0.005	Titanium	0.10
Bismuth	<0.007	Europium	<0.002	Niobium		Scandium	<0.011
Lead	0.033	Samarium	<0.004	Zirconium	0.011	Calcium	27
Thallium	<0.005	Neodymium	<0.004	Yttrium		Potassium	10
Mercury	–[a]	Praseodymium	0.002	Strontium	0.28	Chlorine	0.83
Gold		Cerium	0.003	Rubidium	0.054	Sulphur	56
Platinum		Lanthanum	0.002	Bromine	0.16	Phosphorus	38
Iridium		Barium	0.16	Selenium	0.006	Silicon	48
Osmium	–[b]	Cesium		Arsenic	<0.017	Aluminum	4.6
Rhenium		Iodine	0.002	Germanium		Magnesium	15
Tungsten	<0.009	Tellurium	<0.002	Gallium	0.001	Sodium	490
Tantalum	<0.003[c]	Antimony	0.19	Zinc	1.5	Fluorine	2.9
Hafnium	<0.011	Tin	0.004	Copper	0.49	Oxygen	–[a]
Lutecium	<0.002	Indium	–[b]	Nickel	0.024	Nitrogen	–[a]
Ytterbium	<0.007	Cadmium	0.004	Cobalt	0.021	Carbon	–[a]
Thullium	<0.002	Silver		Iron	2.7	Boron	–[a]
Erbium	<0.005	Palladium		Manganese	0.13	Beryllium	<0.003
Holmium		Rhodium		Chromium	0.015	Lithium	0.004
Dysprosium	<0.002						

Plant G, g/m³

Uranium		Terbium		Ruthenium		Vanadium	0.12
Thorium		Gadolinium		Molybdenum	0.006	Titanium	0.11
Bismuth		Europium		Niobium		Scandium	
Lead	0.10	Samarium		Zirconium	0.011	Calcium	16
Thallium		Neodymium		Yttrium		Potassium	2.0
Mercury	–[a]	Praseodymium		Strontium	0.089	Chlorine	1.1
Gold		Cerium	0.004	Rubidium	0.017	Sulphur	43
Platinum		Lanthanum	0.004	Bromine	0.036	Phosphorus	16
Iridium		Barium	0.23	Selenium	0.001	Silicon	8.7
Osmium	–[b]	Cesium	0.001	Arsenic	0.014	Aluminum	2.2
Rhenium		Iodine	0.007	Germanium		Magnesium	1.4
Tungsten		Tellurium		Gallium		Sodium	48
Tantalum		Antimony	1.2	Zinc	0.84	Fluorine	3.2
Hafnium		Tin	0.032	Copper	0.11	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	0.038	Nitrogen	–[a]
Ytterbium		Cadmium	0.002	Cobalt	0.13	Carbon	–[a]
Thullium		Silver	0.003	Iron	1.6	Boron	–[a]
Erbium		Palladium		Manganese	0.17	Beryllium	
Holmium		Rhodium		Chromium	0.018	Lithium	0.32
Dysprosium							

(continued)

[a] Not reported. [b] Internal standard. [c] Instrument source.

TABLE 22 (continued)

Plant K, g/m^3							
Uranium	<0.002	Terbium		Ruthenium		Vanadium	0.015
Thorium	<0.002	Gadolinium	<0.002	Molybdenum	0.006	Titanium	0.018
Bismuth	<0.004	Europium	≤0.002	Niobium		Scandium	<0.002
Lead	0.017	Samarium	≤0.002	Zirconium	0.005	Calcium	24
Thallium		Neodymium	<0.002	Yttrium		Potassium	26
Mercury	-[a]	Praseodymium		Strontium	0.19	Chlorine	650
Gold		Cerium		Rubidium	0.027	Sulphur	105
Platinum		Lanthanum	<0.002	Bromine	2.9	Phosphorus	6.8
Iridium		Barium	0.19	Selenium		Silicon	120
Osmium		Cesium		Arsenic	0.32	Aluminum	0.71
Rhenium	-[b]	Iodine	23	Germanium	0.010	Magnesium	13
Tungsten	<0.005	Tellurium		Gallium	0.001	Sodium	120
Tantalum	<0.015[c]	Antimony	0.048	Zinc	0.77	Fluorine	87
Hafnium	<0.006	Tin	0.011	Copper	0.11	Oxygen	-[a]
Lutecium		Indium	-[b]	Nickel	0.014	Nitrogen	-[a]
Ytterbium	<0.004	Cadmium	0.003	Cobalt	0.012	Carbon	-[a]
Thullium		Silver		Iron	0.72	Boron	-[a]
Erbium	<0.003	Palladium		Manganese	0.008	Beryllium	<0.002
Holmium		Rhodium		Chromium	0.090	Lithium	0.005
Dysprosium							

Plant L, g/m^3							
Uranium		Terbium		Ruthenium		Vanadium	0.23
Thorium		Gadolinium		Molybdenum	0.015	Titanium	0.24
Bismuth	<0.002	Europium		Niobium	0.005	Scandium	
Lead	0.14	Samarium	≤0.007	Zirconium	0.026	Calcium	110
Thallium		Neodymium		Yttrium	0.001	Potassium	5.3
Mercury	-[a]	Praseodymium		Strontium	0.99	Chlorine	2.1
Gold		Cerium	0.011	Rubidium	0.20	Sulphur	330
Platinum		Lanthanum	0.005	Bromine	0.51	Phosphorus	10
Iridium		Barium	0.37	Selenium	0.002	Silicon	15
Osmium		Cesium	0.007	Arsenic	0.031	Aluminum	0.44
Rhenium	-[b]	Iodine	0.005	Germanium		Magnesium	4.6
Tungsten	<0.003	Tellurium		Gallium		Sodium	78
Tantalum	0.003[c]	Antimony	0.30	Zinc	2.4	Fluorine	2.5
Hafnium	<0.004	Tin	0.046	Copper	0.54	Oxygen	-[a]
Lutecium		Indium	-[b]	Nickel	0.25	Nitrogne	-[a]
Ytterbium	<0.003	Cadmium	0.003	Cobalt	0.033	Carbon	-[a]
Thullium		Silver	0.001	Iron	4.5	Boron	-[a]
Erbium	0.001	Palladium		Manganese	0.27	Beryllium	
Holmium		Rhodium		Chromium	0.26	Lithium	2.1
Dysprosium							

Plant N, g/m^3							
Uranium	<0.004	Terbium		Ruthenium		Vanadium	0.033
Thorium	<0.004	Gadolinium	<0.003	Molybdenum	0.030	Titanium	0.089
Bismuth	<0.008	Europium	<0.003	Niobium	0.001	Scandium	<0.004
Lead	0.95	Samarium	<0.005	Zirconium	0.054	Calcium	570
Thallium	<0.002	Neodymium	<0.004	Yttrium	0.017	Potassium	58
Mercury	-[a]	Praseodymium	0.002	Strontium	2.1	Chlorine	1.1
Gold		Cerium	0.005	Rubidium	0.51	Sulphur	1,400
Platinum		Lanthanum	0.008	Bromine	0.19	Phosphorus	110
Iridium		Barium	1.3	Selenium	0.063	Silicon	54
Osmium		Cesium	0.001	Arsenic	0.40	Aluminum	110
Rhenium	-[b]	Iodine	0.12	Germanium	0.005	Magnesium	42
Tungsten	<0.010	Tellurium		Gallium	0.002	Sodium	150
Tantalum	<0.011	Antimony	0.12	Zinc	580	Fluorine	41
Hafnium	<0.012	Tin	0.008	Copper	0.11	Oxygen	-[a]
Lutecium	<0.002	Indium	-[a]	Nickel	0.39	Nitrogen	-[a]
Ytterbium	<0.008	Cadmium	0.004	Cobalt	0.46	Carbon	-[a]
Thullium	<0.003	Silver	0.002	Iron	80	Boron	-[a]
Erbium	<0.004	Palladium		Manganese	27	Beryllium	<0.004
Holmium		Rhodium		Chromium	44	Lithium	0.033
Dysprosium	<0.002						

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[a] Not reported. [b] Internal standard. [c] Instrument source.

(continued)

TABLE 22 (continued)

Plant S, g/m^3							
Uranium	<0.002	Terbium	≤0.002	Ruthenium		Vanadium	0.006
Thorium	<0.002	Gadolinium		Molybdenum	0.021	Titanium	0.010
Bismuth	0.012	Europium		Niobium	0.002	Scandium	<0.002
Lead	0.085	Samarium		Zirconium	0.016	Calcium	11
Thallium		Neodymium	≤0.004	Yttrium		Potassium	73
Mercury	–[a]	Praseodymium	≤0.005	Strontium	0.027	Chlorine	2.5
Gold		Cerium	≤0.005	Rubidium	0.81	Sulphur	24
Platinum		Lanthanum	≤0.007	Bromine	13	Phosphorus	15
Iridium		Barium	0.097	Selenium	0.001	Silicon	18
Osmium		Cesium	0.002	Arsenic	0.064	Aluminum	18
Rhenium	–[b]	Iodine	0.017	Germanium		Magnesium	2.8
Tungsten	<0.005	Tellurium		Gallium		Sodium	95
Tantalum	<0.012	Antimony	0.84	Zinc	0.29	Fluorine	1.1
Hafnium	<0.004	Tin	0.024	Copper	0.28	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	0.005	Nitrogen	–[a]
Ytterbium	<0.003	Cadmium	0.008	Cobalt		Carbon	–[a]
Thullium	≤0.002	Silver	0.001	Iron	1.0	Boron	–[a]
Erbium	≤0.003	Palladium		Manganese	0.10	Beryllium	<0.002
Holmium		Rhodium		Chromium	0.016	Lithium	0.011
Dysprosium							
Plant T, g/m^3							
Uranium	0.002	Terbium		Ruthenium		Vanadium	0.014
Thorium		Gadolinium		Molybdenum	0.005	Titanium	0.019
Bismuth		Europium		Niobium		Scandium	
Lead	0.042	Samarium		Zirconium	0.006	Calcium	5.2
Thallium		Neodymium		Yttrium	0.002	Potassium	36
Mercury	–[a]	Praseodymium	0.002	Strontium	0.030	Chlorine	0.51
Gold		Cerium	0.002	Rubidium	0.13	Sulphur	0.70
Platinum		Lanthanum	0.006	Bromine	0.13	Phosphorus	0.79
Iridium		Barium	0.022	Selenium	0.005	Silicon	1.9
Osmium		Cesium	0.001	Arsenic	<0.003	Aluminum	3.3
Rhenium	–[b]	Iodine	0.002	Germanium		Magnesium	1.4
Tungsten	<0.002	Tellurium		Gallium		Sodium	40
Tantalum		Antimony	0.009	Zinc	0.29	Fluorine	0.95
Hafnium	<0.002	Tin	0.005	Copper	0.040	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	0.045	Nitrogen	–[a]
Ytterbium	<0.002	Cadmium	0.001	Cobalt	0.002	Carbon	–[a]
Thullium		Silver	0.002	Iron	0.57	Boron	–[a]
Erbium	<0.002	Palladium		Manganese	0.059	Beryllium	
Holmium	≤0.002	Rhodium		Chromium	0.058	Lithium	0.008
Dysprosium							
Plant U, g/m^3							
Utanium		Terbium		Ruthenium		Vanadium	0.002
Thorium		Gadolinium		Molybdenum	0.007	Titanium	0.024
Bismuth	<0.002	Europium		Niobium		Scandium	0.003
Lead	0.006	Samarium		Zirconium	0.002	Calcium	180
Thallium		Neodymium		Yttrium	0.001	Potassium	37
Mercury	–[a]	Praseodymium	≤0.026	Strontium	0.32	Chlorine	170
Gold		Cerium		Rubidium	0.043	Sulphur	16
Platinum		Lanthanum	≤0.081	Bromine	0.55	Phosphorus	6.4
Iridium		Barium	0.16	Selenium	0.018	Silicon	14
Osmium		Cesium	0.002	Arsenic	0.14	Aluminum	0.24
Rhenium	–[b]	Iodine	0.076	Germanium		Magnesium	11
Tungsten	<0.002	Tellurium		Gallium		Sodium	83
Tantalum		Antimony	0.19	Zinc	16	Fluorine	2.4
Hafnium	<0.002	Tin	0.003	Copper	0.099	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	0.058	Nitrogen	–[a]
Ytterbium		Cadmium	0.003	Cobalt	0.10	Carbon	–[a]
Thullium		Silver		Iron	0.12	Boron	–[a]
Erbium		Palladium		Manganese	0.53	Beryllium	
Holmium		Rhodium		Chromium	0.005	Lithium	0.023
Dysprosium							

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[a] Not reported. [b] Internal standard. [c] Instrument source.

(continued)

TABLE 22 (continued)

			Plant V, g/m^3				
Uranium		Terbium		Ruthenium		Vanadium	0.012
Thorium		Gadolinium		Molybdenum	0.005	Titanium	0.12
Bismuth	0.003	Europium		Niobium		Scandium	
Lead	0.089	Samarium		Zirconium	0.002	Calcium	43
Thallium		Neodymium	≤0.002	Yttrium	0.001	Potassium	2.0
Mercury	–[a]	Praseodymium	≤0.002	Strontium	0.19	Chlorine	1.0
Gold		Cerium	0.005	Rubidium	0.022	Sulphur	45
Platinum		Lanthanum	0.003	Bromine	1.1	Phosphorus	4.2
Iridium		Barium	0.36	Selenium		Silicon	9.5
Osmium		Cesium		Arsenic	0.012	Aluminum	4.0
Rhenium	–[b]	Iodine	≤0.003	Germanium		Magnesium	7.4
Tungsten	<0.002	Tellurium		Gallium		Sodium	42
Tantalum		Antimony	0.010	Zinc	3.1	Fluorine	1.4
Hafnium	<0.002	Tin	0.009	Copper	2.0	Oxygen	–
Lutecium		Indium	–[b]	Nickel	0.010	Nitrogen	–
Ytterbium		Cadmium	0.011	Cobalt	0.073	Carbon	–
Thullium	≤0.001	Silver		Iron	4.7	Boron	–
Erbium	≤0.002	Palladium		Manganese	0.31	Beryllium	
Holmium		Rhodium		Chromium	0.066	Lithium	0.041
Dysprosium							
			Plant W, g/m^3				
Uranium	<0.003	Terbium		Ruthenium		Vanadium	0.011
Thorium	≤0.003	Gadolinium	<0.002	Molybdenum	<0.007	Titanium	0.079
Bismuth	≤0.005	Europium	≤0.004	Niobium		Scandium	<0.002
Lead	0.17	Samarium	≤0.003	Zirconium	0.023	Calcium	94
Thallium	<0.002	Neodymium	≤0.004	Yttrium	0.006	Potassium	660
Mercury	–[a]	Praseodymium	≤0.004	Strontium	0.31	Chlorine	1.2
Gold		Cerium	≤0.010	Rubidium	2.0	Sulphur	16
Platinum		Lanthanum	≤0.006	Bromine	0.55	Phosphorus	0.12
Iridium		Barium		Selenium	0.018	Silicon	30
Osmium		Cesium	0.003	Arsenic	0.033	Aluminum	7.3
Rhenium	–[b]	Iodine	≤0.013	Germanium	<0.004	Magnesium	12
Tungsten	<0.007	Tellurium		Gallium	0.004	Sodium	8.3
Tantalum	<0.003[c]	Antimony	≤0.013	Zinc	0.060	Fluorine	11
Hafnium	≤0.009	Tin	0.011	Copper	0.072	Oxygen	–[a]
Lutecium	≤0.001	Indium	–[b]	Nickel	0.024	Nitrogen	–[a]
Ytterbium	<0.006	Cadmium	0.006	Cobalt	0.048	Carbon	–[a]
Thullium	<0.002	Silver	0.003	Iron	0.67	Boron	–[a]
Erbium	<0.003	Palladium		Manganese	0.22	Beryllium	<0.003
Holmium		Rhodium		Chromium	0.028	Lithium	0.054
Dysprosium	≤0.001						
			Plant X, g/m^3				
Uranium		Terbium		Ruthenium		Vanadium	0.026
Thorium		Gadolinium		Molybdenum	0.005	Titanium	0.018
Bismuth		Europium		Niobium		Scandium	<0.002
Lead	0.030	Samarium		Zirconium		Calcium	23
Thallium		Neodymium		Yttrium	0.007	Potassium	9.0
Mercury	–[a]	Praseodymium		Strontium	0.14	Chlorine	130
Gold		Cerium		Rubidium	0.023	Sulphur	29
Platinum		Lanthanum	0.001	Bromine	2.5	Phosphorus	29
Iridium		Barium	0.13	Selenium		Silicon	18
Osmium		Cesium		Arsenic	0.022	Aluminum	8.2
Rhenium	–[b]	Iodine	0.037	Germanium	0.005	Magnesium	4.6
Tungsten	<0.002	Tellurium		Gallium	0.001	Sodium	33
Tantalum		Antimony	1.9	Zinc	0.14	Fluorine	11
Hafnium	<0.002	Tin	0.004	Copper	0.87	Oxygen	–[a]
Lutecium		Indium	–[b]	Nickel	0.006	Nitrogen	–[a]
Ytterbium	<0.002	Cadmium		Cobalt	0.12	Carbon	–[a]
Thullium		Silver		Iron	2.5	Boron	–[a]
Erbium		Palladium		Manganese	0.028	Beryllium	
Holmium		Rhodium		Chromium	0.027	Lithium	0.052
Dysprosium							

[a] Not reported. [b] Internal standard. [c] Instrument source.

TABLE 23. CONCENTRATION OF METHYLENE CHLORIDE EXTRACTABLE ORGANICS IN FILTERED SECONDARY EFFLUENTS

Plant	Organic concentration, g/m^3	Plant	Organic concentration, g/m^3
A	63.7	N	9.24
B	3.18	S	5.40
C	28.2	T	17.8
E	3.60	U	14.6
F	16.0	V	a
G	27.2	W	15.0
K	2.73	X	13.5
L	18.3		

[a] Analysis not performed.

The methylene chloride extract then went through a solvent exchange step to transfer the sample to a nonpolar solvent. This extract was then passed through a liquid chromatography column that divided the sample into eight fractions. Each of the eight fractions was analyzed by an infrared (IR) spectrophotometer and then by a low resolution mass spectrometer (LRMS). The classes of organic compounds and their relative intensities found in each fraction are presented in Tables 24 and 25.

IR analyses indicated the presence of aliphatic hydrocarbons, C=O esters and acids, aromatics, phthalate esters, and fatty acid groups. LRMS analyses identified the following major classes of compounds: paraffinic/olefinic, alkyl benzenes, alcoholic ethers, di-n-octyl phthalate, bis(hydroxy-t-butyl phenol) propane, tri-t-butyl benzene, alkyl phenols, dichloroaniline, toluene-sulfonyl groups, vinyl stearate, and azo compounds.

TABLE 24. LEVEL 1 INFRARED ANALYSIS OF THE ORGANIC EXTRACTS

Fraction No.	Interpretation
	Plant A
Before extraction	Bonded OH, aliphatic CH, C=O ester and acid, ketone or aldehyde, conjugated C=C, possible aromatic C=C, ether groups $(CH_2)_n$, where n = ≥ 4
1	All aliphatic hydrocarbons
2	Aliphatic and aromatic hydrocarbons, aromatic C=C
3	Aliphatic C-H, ester or aldehyde C=O, conjugated C=C, various CH_2 groups, or aromatic substitution bonds
4	Similar to fraction 3
5	Similar to fraction 3
6	Bonded OH; aliphatic C-H; acid, ketone, or aldehyde C=O; conjugated and aromatic C=C; possible phthalate ester; ether group or Si-O
7	Bonded OH, aliphatic CH, ester or aldehyde C-O, water in material, ether or Si-O groups
8	Bonded OH and 1,630 cm^{-1} absorption-water, aliphatic CH trace of C=O, SiO_2, poorly defined organic
	Plant B
Before extraction	Bonded OH, aliphatic CH, ether, $(CH_2)_4$, C=O, or C=C?
1	Aliphatic CH, trace aromatic CH, $Si-CH_3$(?), methylene CH_2 groups >4
2	Very strong background adsorption--only aliphatic CH visible
3	Bonded OH, aliphatic CH, ester C=O, acid, aldehyde or ketone C=O $(CH_2)_n$ or >4.
4	Bonded OH, trace aromatic CH, aliphatic CH, ester C=O acid, aldehyde or ketone C=O, various CH_2 groups.
5	Similar to 4, but stronger bonded OH, less ester C=O, more acid, aldehyde or ketone C=O, various CH_2 groups complex spectrum.
6	Bonded OH, aliphatic CH, some ester C=O, acid, aldehyde or ketone C=O, ether groups, may contain glycol ether type compounds.
7	Bonded OH, aliphatic CH, ester C=O, acid, aldehyde or ketone C=O, strong ether group, glycol ether type of compound.
8	Strong bonded OH, weak C-H (aliphatic) trace C=O, nonconjugated C=C, SiO_2 present.
	Plant E
Before extraction	Aliphatic CH, diffuse C=O region, carboxylate ion, ether group complex spectrum
1	Aliphatic hydrocarbons, no indication of number of CH_2 groups.
2	Poor spectrum--aliphatic CH, CO_2, and water vapor in spectrum.
3	Poorly defined spectrum--aliphatic CH, numerous ill-defined bonds. No C=O or C=C.
4	Aliphatic CH, ester C=O, strong background adsorption.
5	Aliphatic CH, ester=O, most likely aliphatic ester, possibly acetate--may be single compound.
6	Spectrum too strong--bonded OH, aliphatic CH, ester C=O, acid, ketone or aldehyde C=O, large portion of compound in No. 5 plus additional carbonyl compounds.
6 (repeat)	Mixture of several comps. Ester C=O, and acid, ketone or aldehyde C=O. Ether group.
7	Bonded OH, aliphatic CH, acid, ketone or aldehyde C=O, spectrum too strong for good identification.
7 (repeat)	Bonded OH, aliphatic CH, acid, aldehyde or ketone C=O, evidence of both acid and acid salt (carboxylate ion) $CH(CH_3)_2$ group possible, no long chain $(CH_2)_n$ groups.
8	Bonded OH--evidence of water (3,350 cm^{-1} and 1,635 cm^{-1}) SiO_2 present aliphatic CH.

(continued)

TABLE 24 (continued)

Fraction No.	Interpretation
	Plant F
Before extraction	Bonded OH, aliphatic CH, diffuse C=O, and C=C regions Si-O possible, trace CH_2Cl_2.
1	Long chain aliphatic hydrocarbons. Unknown 1,265 cm^{-1} bond. $Si-(CH_3)$-?
2	Aliphatic CH, ester C=O, conjugated C=C, trace 1,265 cm^{-1}.
3	Weak bonded OH, aliphatic CH, aromatic CH-?, ester C=O, conjugated C=C, progression of CH substitution bonds, series of CH_2 bond.
4	Similar to No. 3, series of ill-defined bonds below 1,400 cm^{-1}.
5	Bonded OH, trace aromatic CH, aliphatic CH, ester or aldehyde C=O, conjugated C=C, series of CH_2 groups aromatic substitution bonds-?
6	Bonded OH, aliphatic CH, ester C=O, nonconjugated C=C or amides, ether or SiO groups, secondary amide possible, CH_2 groups n >4.
7	Similar to No. 6.
8	Strong bonded OH, aliphatic CH, weak ester or aldehyde C=O, H_2O present (1,640 cm^{-1}) SiO_2 present.
	Plant G
Before extraction	Aromatic and aliphatic CH, residual CH_2Cl_2 in spectrum.
1	Aliphatic hydrocarbon--chain length >C_4, possible $C(CH_3)_3$ group.
2	Aliphatic CH, ester C=O, phthalate bonds, various chain lengths of CH_2.
3	Aliphatic CH, ester C=O, some C=C, various CH_2 groupings.
4	Bonded O-H, aliphatic CH, ester C=O, some acid, aldehyde or ketone C=O, C=C, possible fatty acid group, various CH_2 groups.
5	Identical to No. 4.
6	Considerable bonded OH, aliphatic CH, ester C=O conjugated C=C, Si-O or ether group.
7	Similar to No. 6.
8	Considerable O-H, aliphatic C-H, ester C=O, SiO or ether groups.
	Plant K
Before extraction	Bonded OH, aliphatic CH, diffuse C=O region $Si(CH_3)$ group? Diffuse spectrum.
1	Appears to contain water, aliphatic C-H, $Si-CH_3$, mainly hydrocarbon compounds.
2	Aliphatic hydrocarbons, silicones.
3	Mainly silicone type materials.
4.	Bonded OH, some aromatic CN, aliphatic CH, ester C=O, acid, ketone or aldehyde C=O, carboxylate ion, conjugated C=C, some silicone material, $(CH_2)_n$ where n >4.
5	Strong background adsorption, aliphatic CH, ester C=O various $(CH_2)_n$ groups, some silicone adsorption.
6	Bonded OH, aliphatic CH, trace aromatic CH, ester C=O, aromatic C=C, silicone adsorption.
7	Similar to No. 6, not as strong a spectrum.
8	Strong OH adsorption, very weak C-H, trace of C=O (may be H_2O background) SiO_2 adsorption, low organic content.

(continued)

TABLE 24 (continued)

Fraction No.	Interpretation
	Plant L
Before extraction	Bonded OH, aliphatic CH, C=O, aromatic or conjugated C=C, ether group. Complex spectrum $C\equiv N$, SiO_2-?
1	Aliphatic CH, trace C=O, C=C, CH_2 group >4, possible $CH(CH_3)_2$ group--mainly aliphatic hydrocarbons.
2	Aliphatic hydrocarbons--branched chain, trace of C=O, C=C no long $(CH_2)_n$ groups.
3	Bonded OH, aliphatic CH, ester C=O, conjugated C=C strong ether group, possible glycol ether.
4	Bonded OH, aliphatic CH, ester C=O, nitrite ($C\equiv N$) group, strong ether group, various streight and branched CH_2 groups.
5	Bonded OH, some aromatic CH, aliphatic CH, $C\equiv N$ nitrite group, ester C=O, conjugated or aromatic C=C, ether group, very complex mixture spectrums.
6	Similar to No. 5 but less $C\equiv N$, more bonded OH, ester C=O, conjugated or aromatic C=C, some aromatic C-H, aliphatic CH ether grouping--complex spectra.
7	Some bonded OH, aliphatic CH, trace $C\equiv N$, ester C=O, carboxylate ion, possible C-Cl group.
8	Contain water and SiO_2, plus some of materials found in No. 7. Carboxylate ion.
	Plant N
Before extraction	Bonded OH, aliphatic CH, diffuse C=O, C=C region, silicone $(CH_2)_4$ ether?
1	Long chain aliphatic hydrocarbon.
2	Aliphatic CH, ester C=O, possible $(CH(CH_3)_2$ group, spectrum not very distinct.
3	Weak bonded OH, aliphatic CH, ester C=O, conjugated C=C, $CH(CH_3)_2$ group, various CH_2 groups.
4	Weak bonded OH, aliphatic CH, ester C=O, possible fatty acid groups.
5	Bonded OH, aliphatic CH, medium ester C=O, nonconjugated C=C, spectrum not very distinct.
6	Bonded OH, aliphatic CH, ester C=O, acid, aldehyde or ketone C=O, conjugated C=C, aliphatic ketone or ester group, possible ether group, various CH_2 chain lengths.
7	Bonded OH, aliphatic CH, ester C=O, conjugated C=C, possible fatty acid gropus, ether group.
8	Strong OH, weak aliphatic CH, ketone, acid or aldehyde C=O, ester C=O, strong C=C, ether group.
	Plant S
Before extraction	Bonded OH, aliphatic CH, ester C=O, $(CH_2)_n$ groups, broad diffuse spectrum, ether groups possible.
1	Aliphatic hydrocarbon, chain length $<C_4$ unidentified bond at 1,265 cm^{-1}.
2	Aliphatic hydrocarbons, trace of ester C=O, trace of C=C, not well defined below 1,300 cm^{-1}.
3	Aliphatic CH, trace of aromatic CH, very strong ester C=O or aldehyde C=O. Ester group may be acetate; if aldehyde, long chain aldehyde.
4	Strong background absorption 4,000 cm^{-1} to 900 cm^{-1}. Aliphatic CH, ester or aldehyde C=O. Poor spectrum for interpretation.
5	Nearly identical to fraction No. 4.
6	Very complex spectra. Bonded OH, aliphatic CH, ester and acid, ketone or aldehyde C=O, ether or SiO groups. Several types of CH_2 groupings.
7	Weak and diffuse spectra. Aliphatic CH, ester C=O serve C=C, ether or SiO group.
8	Strong OH group, weak aliphatic CH, weak acid; aldehyde or ketone C=O, strong conjugated C=C contains some SiO_2, diffuse spectrum.

(continued)

TABLE 24 (continued)

Fraction No.	Interpretation
	Plant T
Before extraction	Bonded OH, aliphatic CH, C=O, C=C, ether of SiO groups, $(CH_2)_n$ groups. Very diffuse spectrum.
1	Bonded OH, aliphatic CH, ester C=O, some C=C, CH_2 groups $>C_4$.
2	Bonded OH, aliphatic CH, medium ester C=O, possible fatty acid groups, no definite CH_2 groupings.
3	Bonded OH, aliphatic CH, strong ester C=P, nonconjugated C=C, ether or SiO groups. Various chain lengths of $(CH_2)_n$.
4	Bonded OH, medium aliphatic CH, medium ester C=O, nonconjugated C=C, ether or SiO group, various $(CH_2)_n$ groups, possible acid salts.
5	Similar to No. 3.
6	Bonded OH, aliphatic CH, medium ester C=O, series of 5 unknown bonds medium intensity 1,510 cm^{-1} to 1,610 cm^{-1}, ether or SiO group, various $(CH_2)_n$ groups.
7	Similar to No. 6, but weaker OH, C=CO, conjugated C=C, fatty acid groups? ether or SiO group. $(CH_2)_n$ groups.
8	Very strong bonded OH group, medium aliphatic CH, weak ester C=O, strong nonconjugated C=C, possible fatty acid group, ether or SiO group, no CH_2 $>C_4$.
	Plant U
Before extraction	Trace bonded OH, aliphatic CH, acid, ketone or aldehyde C=O, silicones, some $(CH_2)_n$ groups.
1	Aliphatic hydrocarbon, conjugated or aromatic C=C, weak, $(CH_2)_n$--no >4.
2	Aliphatic hydrocarbon, conjugated C=C (alkene?), diffuse CH_2 groups.
3	Aliphatic C-H, ester C=O, conjugated or aromatic C=C, possible unsaturated ester-fumarate, maleate, etc.
4	Bonded OH, trace sec N-H or oxcetone C=O, aliphatic C-H possible trace $C\equiv N$, ester C=O, conjugated C=C, unsaturated ester group. Complex spectra below 1,500 cm^{-1}.
5	Aliphatic C-H, ester C=O, diffuse spectra below 1,100 cm^{-1}.
6	Complex spectra, bonded OH, NH or C=O overtone, aliphatic C-H, ester C=O, acid, aldehyde or ketone C=O (weak), ether group, complex bond pattern below 1,500 cm^{-1}.
7	Bonded OH, aliphatic C-H, ester C=O, conjugated C=C, possible ether group, miscellaneous $(CH_2)_n$ groups.
8	Bonded OH strong, aliphatic CH, strong C=C, ether group, some SiO_2, possible glycol ethers.
	Plant V
Before extraction	Trace bonded OH, aliphatic CH, ester C=O, acid, aldehyde or ketone C=O, silicone adsorption, some $(CH_2)_n$ groups.
1	Aliphatic CH, $(CH_2)_n$ where n >4, hydrocarbons plus possible $Si-CH_3$.
2	Aliphatic and aromatic CH, some ester C=O, silicones.
3	Poor spectrum--low organic content? aliphatic CH strong background adsorption.
4	Bonded OH, aromatic and aliphatic CH, ester C=O, conjugated C=C, silicones.
5	Identical to No. 4.
6	Aliphatic CH, ester C=O, silicones, CH_2 various groups ester stronger, silicones weaker than in No. 4 or No. 5.
7	Bonded OH, aliphatic CH, ester C=O, conjugated C=C ether group--possible glycol ether-type compounds.
8	Strong bonded OH, weak aliphatic CH, ester C=O, strong C=C, some ether under SiO_2 adsorption.

(continued)

TABLE 24 (continued)

Fraction No.	Interpretation
	Plant W
Before extraction	Bonded OH, aliphatic CH, ester C=O, acid, ketone or aldehyde C=O. $(CH_2)_n$-n where n >4, diffuse spectrum 1,300 cm^{-1} to 900 cm^{-1}.
1	Bonded OH, aliphatic CH, ester C=O, possible ether group $(CH_2)_n$ where n >4. Not typical fraction 1.
2	Aliphatic CH groups, ester C=O, weak spectrum.
3	Aliphatic ester compounds, ester C=O, no aromatic CH.
4	Trace OH, aliphatic CH, ester C=P, weak diffuse spectrum.
5	Weak diffuse spectrum, poor background, OH (water?), aliphatic CH, weak ester C=O, nonconjugated C=C.
6	Bonded OH, aliphatic CH, ester C=O, long chain CH_2 groups, unsaturated acid, aldehyde or ketone, conjugated C=C, possible amide groups.
7	Poor spectrum. Bonded OH, aliphatic CH, numerous C=O types, C=C, amide possible, very diffuse below 1,400 cm^{-1}.
8	Weak spectrum, bonded OH--likely H_2O, very weak aliphatic CH, low organic content.
	Plant X
Before extraction	Bonded OH, aliphatic CH, C=O, ether or SiO, $(CH_2)_n$, numerous broad diffuse bonds.
1	Aliphatic hydrocarbons, possible $Si(CH_3)$.
2	Aliphatic CH, ester C=O, conjugated C=C, hydrocarbon.
3	Bonded OH, aliphatic ch, ester C=O, phthalate plus other types of ester materials.
4	Similar to fraction No. 3.
5	Bonded OH, trace aromatic CH, aliphatic CH, ester C=O, acid, aldehyde or ketone C=O, conjugated C=C, various straight and branched CH_2 chains.
6	Bonded OH, aliphatic CH, ester C=O, conjugated and aromatic C=C, ether group, long CH_2 chains.
7	Very similar to No. 6.
8	Strong OH adsorption, similar to No. 6 and No. 7 but more OH and presence of SiO_2.

TABLE 25. LEVEL 1 LOW RESOLUTION MASS SPECTROMETER ANALYSIS OF ORGANIC FRACTIONS

Plant and organic fraction	Fraction weight, mg	Categories present: Relative intensity[a]	Categories present: Category	Subcategories present: Relative intensity	Subcategories present: Specific compounds	Other unknown compounds present
					Plant A	
1	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
2	16.1	100	Aliphatics[c]	100	Paraffinic/olefinic (or cyclic-paraffinic, etc.).[d,e]	No masses above 490.
		100	Aromatics	100	Alkyl benzenes (91, 105, 114, 133 ions present.[d,e]	
3	5.3	100	Aliphatics	100	Paraffinic/olefinic (or cyclic-paraffinic, etc.).[d,e]	10:368(100), 369(45), 353(20)
		100	Aromatics	100	Alkyl benzenes (91, 105, 114, 133 ions present).[d,e]	1:345(100), 396(35), 411(30)
4	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
5	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
6	10.2	100	Aliphatics	100	Paraffinic/olefinic (or cyclic-paraffinic, etc.).[d,e]	No masses above 414.
		10	Alcohols/ethers	10	Alcoholic ethers (45 ions to 89 ions).[d,e]	
		10	Phenols	10	Bis(hydroxy-t-butyl phenyl) propane ($C_{23}H_{32}O_2$) MW[f] 340.	
		10	Esters	10	Di-n-octyl phthalate ($C_{24}H_{38}O_4$) MW390.	
7	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
8	21.0	10	Aliphatics	10	Paraffinic/olefinic (or cyclic-paraffinic, etc.).[d,e]	No masses above 368. 100:254(100), 126(20), 127(15)
		100	Alcohols/ethers	100	Alcoholic-ethers (45 ions to 89 ions).[d,e] Ethers: Di-n-octyl phthalate ($C_{24}H_{38}O_4$) MW390.	
					Plant B	
1	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
2	10.3	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 354.
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
3	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
4	3.8	10	Aliphatic	10	Same as Plant A, Fraction 2.	No masses above 483. 10:279(100), 294(28), 280(12) 100:341(100), 356(36), 342(27) 100:381(100), 396(15), 382(11) 100:410(100), 151(37), 411(31) 100:429(100), 444(23), 445(15)
5	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
6	13.9	1	Aliphatics	1	Same as Plant A, Fraction 2.	No masses above 437.
		100	Phenols	100	Bis(hydroxy-t-butyl phenol) propane ($C_{23}H_{32}O_2$) MW390.	
7	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
8	15.0	10	Aliphatics	10	Same as Plant A, Fraction 2.	No masses above 414. 100:294(100), 127(20), 128(12)
		10	Alcohols/ethers	10	Alcoholic-ethers (48 ions to 89 ions).[d,e]	
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
					Plant F	
1	9.4	100	Aliphatics	100	Primarily paraffinic.[d,e]	No masses above 446.
2	0.3	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 354.
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
3	1.9	1	Aliphatics	1	Aliphatics.[d,e]	No masses above 354.
		100	Aromatics	100	Alkyl benzenes: Tri-t-butyl benzene ($C_{18}H_{30}$) MW246.	
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
4	3.3	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 381.
		10	Aromatics	10	Tri-t-butyl benzene ($C_{18}H_{30}$) MW246.	
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
5	1.4	10	Aliphatics	10	Same as Plant A, Fraction 2.	No masses above 340. 10:239(100), 240(20), 254(25)
		1	Aromatics	1	Alkyl benzenes: Tri-t-butyl benzene ($C_{18}H_{30}$) MW246.	
		10/100	Phenols	10	Alkyl phenols (135, 107, 121, 148 ions).[d,e]	
				100	Bis(hydroxy-t-butyl phenyl) propane ($C_{23}H_{32}O_2$) MW390.	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
6	18.6	1	Aromatics	1	Tri-t-butyl benzene ($C_{18}H_{30}$) MW246.	No masses above 354. 100:45(100), 42(90) (2 compounds)
		10/100	Phenols	10	Alkyl phenols (135, 107, 121, 149 ions).[d,e]	
				100	Bis(hydroxy-t-butyl phenyl) propane ($C_{23}H_{32}O_2$) MW 390.	
		10	Esters	10	Same as esters, Plant A, Fraction 6.	100:59(100) (4 compounds)
7	51.7	100	Aliphatic	100	Primarily paraffinic.[d,e]	No masses above 354.
		10	Phenols	10	Bis(hydroxy-t-butyl phenyl) propane ($C_{23}H_{32}O_2$) MW390.	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
8	42.7	10	Aliphatics	10	Same as Plant A, Fraction 2.	No masses above 354.
		100	Esters	100	Same as esters, Plant A, Fraction 6.	

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[a] Relative intensity of the mass-to-charge ratio (intensity relative to dominant ion).

[b] Organic weight of fraction below gravimetric threshold of 0.1 mg; therefore, no analysis was performed.

[c] Generally all ions up through 490 present in aliphatic-type pattern; however, all mass >100 are abnormally strong for typical aliphatics.

[d] No molecular weight range determination possible.

[e] No composition determination possible.

[f] Molecular weight.

(continued)

TABLE 25 (continued)

Plant and organic fraction	Fraction weight, mg	Categories present		Subcategories present		Other unknown compounds present
		Relative intensity[a]	Category	Relative intensity	Specific compounds	
					Plant G	
1	31.8	100	Aliphatics	100	Same as Plant F, Fraction 2.	No masses above 410.
2	7.2	1	Aliphatics	1	Same as Plant A, Fraction 2.	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
3	2.3	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 378.
		1	Esters	1	Same as esters, Plant A, Fraction 6.	
4	0.9	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 381.
		1	Esters	1	Same as esters, Plant A, Fraction 6.	
5	0.3	10	Aliphatics	10	Same as Plant A, Fraction 2.	No masses above 325.
		1	Phenols	1	Same as phenols, Plant A, Fraction 6.	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
6	29.0	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 354. 1:69(100), 41(87), 43(78)
		100	Alcohols/ethers	100	Alcoholic ethers with 41, 43, 45 ions and 55, 57, 59 ions.[d,e]	
		1	Phenols	1	Same as phenols, Plant A, Fraction 6.	
		1	Esters	1	Same as esters, Plant A, Fraction 6.	
7	13.9	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 354. 10:69(100), 41(80), 43(78)
		100	Alcohols/ethers	100	Same as Fraction 6.	
		1	Phenols	1	Same as phenols, Plant A, Fraction 6.	
		1	Esters	1	Same as esters, Plant A, Fraction 6.	
8	15.5	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 354.
		10	Alcohols/ethers	10	Same as Fraction 6.	
		1	Esters	1	Same as esters, Plant A, Fraction 6.	
					Plant L	
1	12.7	100	Aliphatics	100	Same as Plant F, Fraction 1.	No masses above 367.
2	2.2	100	Aliphatics	100	Same as Plant F, Fraction 1.	No masses above 367.
		100	Aromatics	100	Same as Plant A, Fraction 3.	
3	8.6	100	Phenols	100	Alkyl phenols (135, 107, 121, 149 ions).[d,e]	No masses above 354. 10:69(100), 41(80), 43(78)
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
4	8.6	100	Aliphatics	100	Same as Plant F, Fraction 1.	No masses above 429.
		100	Aromatics	100	Same as Plant F, Fraction 3.	
		100	Phenols	100	Di-t-butyl phenol ($C_{14}H_{22}O$) MW206.	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
5	9.7	100	Aliphatics	100	Same as Plant F, Fraction 1.	No masses above 429. 10:69(100), 41(80), 43(78)
		100	Phenols	100	Alkyl phenol (135, 107, 121, 149 ions).[d,e]	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
6	55.1	100	Phenols	100	Alkyl phenols (135, 107, 121, 149 ions).[d,e]	No masses above 340.
		100	Esters	100	Phthalate, probably di-C_8 alkyl but with a new series of ion added (223, 237, 251, 265, 279).	
7	11.7	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 381. 100:69(100), 41(80), 43(78) 1:158 1:200(100), 201(86) 10:280(100), 279(98)
		100/10	Phenols	100	Alkyl phenols (135, 107, 121, 144 ions).[d,e]	
				10	Bis(hydroxy-t-butyl phenyl) propane ($C_{23}H_{32}O_2$) MW340.	
		100	Esters	100	Same as Plant A, Fraction 6.	
8	18.1	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 279. 1:Cesium iodide from infrared plates.
		100	Esters	100	Same as esters, Plant A, Fraction 6.	

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[a] Relative intensity of the mass-to-charge ratio (intensity relative to dominant ion).

[d] No molecular weight range determination possible.

[e] No composition determination possible.

(continued)

TABLE 25 (continued)

Plant and organic fraction	Fraction weight, mg	Categories present: Relative intensity[a]	Categories present: Category	Subcategories present: Relative intensity	Subcategories present: Specific compounds	Other unknown compounds present
Plant N						
1	44.5	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 428.
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
2	1.0	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 394.
		100	Aromatics	100	Alkyl benzene (91, 105, 119 ions).[d,e]	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
3	3.9	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 498. 10:368(100), 369(30), 353(20) 10:395(100), 396(30) 1:410(100), 411(30)
		100	Aromatics	100	Same as Plant A, Fraction 3.	
		10	Esters	10	Same as esters, Plant A, Fraction 6	
4	2.1	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 499. 10:368(100), 369(30), 353(20) 10:395(100), 396(30) 10:410(100), 411(30)
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
5	1.0	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 279.
		10	Phenols	10	Same as Plant F, Fraction 5.	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
6	20.5	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 490. 1:155
		10	Aromatics		t-Butyl dichlorobenzene ($C_{10}H_{12}Cl_2$) MW202	
		10	Amines	10	Dichloroaniline ($C_6H_5NCl_2$) MW 161	
		10/10	Phenols	10	Same as phenols, Plant F, Fraction 5.	
				10	Same as phenols, Plant A, Fraction 6.	
		10	Esters	10	Phthalate, probably di-C_8 alkyl but with a new series of ions added: 223, 237, 251, 265, 279 ($C_{24}H_{38}O_4$) MW 390 (probably).	
7	8.9	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 340.
		10/1	Phenols	10	Same as phenols, Plant F, Fraction 5.	
				1	Same as phenols, Plant A, Fraction 6.	
		1	Esters	1	Same as esters, Plant A, Fraction 6.	
8	16.6	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 279. 1:Cesium iodide.
		10	Phenols	10	Same as phenols, Plant F, Fraction 5.	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
Plant S						
1	12.3	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 446.
2	4.7	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 446.
		100	Aromatics	100	Same as Plant A, Fraction 2.	
3	19.7	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 452.
		10	Aromatics	10	Same as Plant F, Fraction 3.	
		1	Esters	1	Same as Plant A, Fraction 6.	
4	11.1	10	Aliphatics	10	Same as Plant A, Fraction 2.	No masses above 477. 1:381(100), 382(27), 396(17)
		100/10	Aromatics	100	Toluene-sulfonyl group (91, 155 ions). Best identity is *p*-toluene sulfonamide.	
		10	Phenols	10	Same as Plant F, Fraction 3.	
		10	Phenols	10	Di-t-butyl phenol ($C_{14}H_{22}O$) MW206.	
5	6.6	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 354. 100:98(100), 97(75)
		100	Aromatics	100	Toluene-sulfonyl group (91, 155 ions). Best identity *p*-toluene sulfonamide.	
6	26.8	1	Aliphatics	1	Same as Plant A, Fraction 2.	No masses above 446. 10:90(100), 91(60), 106(58)
		100	Aromatics	100	Toluene-sulfonyl group (91, 155 ions).[d,e]	
		10	Phenols	10	Same as phenols, Plant A, Fraction 6.	
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
7	6.5	1	Aliphatics	1	Same as Plant A, Fraction 2.	No masses above 446. 10:90(100), 91(60), 106(58)
		10	Aromatics		Same as aromatics, Plant A, Fraction 4.	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
8	13.6	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 354.
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
				1	Methyl esters (74, 87 ions).[d,e]	

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[a] Relative intensity of the mass-to-charge ratio (intensity relative to dominant ion).

[d] No molecular weight range determination possible.

[e] No composition determination possible.

(continued)

TABLE 25 (continued)

Plant and organic fraction	Fraction weight, mg	Categories present		Subcategories present		Other unknown compounds present
		Relative intensity[a]	Category	Relative intensity	Specific compounds	
					Plant T	
1	18.5	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 362.
		1	Esters	1	Same as esters, Plant A, Fraction 6.	
2	2.6	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 312. 10:69(100), 41(80), 43(78)
		100	Aromatics	100	Same as Plant A, Fraction 2.	
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
3	6.7	100/100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 486. 1:314(100), 315(25) 10:410(100), 411(32)
				100	Same as Plant F, Fraction 1.	
		10	Aromatics	10	Same as Plant F, Fraction 3.	
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
4	12.9	100	Aliphatics	100	Same as Plant F, Fraction 1.	No masses above 396. 10:69(100), 41(80), 43(78)
		10	Aromatics	10	Same as Plant F, Fraction 3.	
		10	Phenols	10	Di-t-butyl phenol ($C_{14}H_{22}O$) MW 206.	
		1	Esters	1	Same as Plant A, Fraction 6.	
5	6.9	10	Aliphatics	10	Same as Plant A, Fraction 2.	No masses above 396.
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
6	17.4	100	Aliphatics	100	Same as Plant F, Fraction 1.	No masses above 340. 100:99
		100/100	Phenols	100	Same as Plant F, Fraction 3.	
				100	Same as phenols, Plant A, Fraction 6.	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
7	29.2	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 279.
		100	Phenols	100	Same as phenols, Plant F, Fraction 5.	
		100	Esters	100	Phthalate, probably di-C_8 alkyl but with a new series of ions added: 223, 237, 251, 265, 279 ions ($C_{24}H_{38}O_4$) MW 390.	
8	17.1	10	Aliphatics	10	Same as Plant A, Fraction 2.	No masses above 336. 100:117(100), 59(44) 10:69(100), 41(80), 43(78) Also many organo-silicon ions; e.g., 207, 221, etc.
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
					Plant U	
1	59.1	100	Aliphatics	100	Same as Plant F, Fraction 1.	No masses above 404.
2	8.1	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 410.
		100	Aromatics	100	Same as Plant A, Fraction 2.	
3	11.0	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 398 in aliphatic-type pattern; all masses above 100 are abnormally strong for typical aliphatics.
4	4.4	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 411. 10:410(100), 411(30)
		100/10	Esters	100	Same as esters, Plant A, Fraction 6.	
				10	Vinyl stearate ($C_{20}H_{38}O_2$) MW310	
		10	Amines	10	Azo compounds: naphthalane-mno-ago-benzene ($C_{16}H_{12}ON_2$) MW248.	
5	5.3	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 444. 10:429(100), 444(20)
		100	Esters	100	Some as esters, Plant A, Fraction 6.	
		10	Amines	10	Halogenated amines: chloroaniline (C_6H_6Cl) MW127.	
6	21.9	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 496.
		100	Esters	100	Same as esters, Plant A, Fraction 6, and vinyl stearate ($C_{20}H_{38}O_2$) MW310.	
		10	Amines	10	Chloroaniline (C_6H_6Cl) MW 127.	
7	11.5	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 495. Similar to unusual pattern in Fraction 3 through mass 495.
		100	Phenols	100	Same as phenols, Plant A, Fraction 6.	
		10/100	Esters	10	Same as esters, Plant A, Fraction 6.	
				100	Vinyl stearate ($C_{20}H_{38}O_2$) MW 310.	
8	23.8	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 340. 100:155 10:254, 127 (diatomic iodine or probably napthyl iodide). 1:Cesium iodide.
		10	Esters	10	Same as esters, Plant A, Fraction 6.	

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[a] Relative intensity of the mass-to-charge ratio (intensity relative to dominant ion).

[d] No molecular weight range determination possible.

[e] No composition determination possible.

(continued)

TABLE 25 (continued)

Plant and organic fraction	Fraction weight, mg	Categories present		Subcategories present		Other unknown compounds present
		Relative intensity[a]	Category	Relative intensity	Specific compounds	
Plant V						
1	15.3	100	Aliphatics	100	Same as Plant F, Fraction 1.	No masses above 451.
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
2	4.9	100	Organo-silicon species	100	73(dominant), 147, 207, 221, 355 ions.[d,e]	No masses above 491.
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
3	3.8	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 296. 10:69(100), 41(80), 43(78)
		1	Aromatics	1	Same as Plant A, Fraction 2.	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
4	5.5	10	Aliphatics	10	Same as Plant F, Fraction 1.	No masses above 477. 100:69(100), 41(80), 43(78)
		10	Organo-silicon species	10	73(dominant), 147 ions.[d,e]	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
5	11.8	10	Phenols	10	Same as phenols, Plant A, Fraction 6.	No masses above 340. 10:69(100), 41(80), 43(78)
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
6	3.9	100	Aliphatics	100	Same as Plant F, Fraction 1.	No masses above 253. 10:156(100), 155(35) (Possibly bipyridyl or phenyl cyclohexadiene, MW156 each)
		100	Phenols	100	Same as phenols, Plant A, Fraction 6.	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
7	22.6	10	Aliphatics	10	Same as Plant F, Fraction 1.	No masses above 373.
		100	Alcohols/ethers	100	Pattern indicates alcoholic ethers: 41, 43, 49 (dominant) and 55, 57, 59 (dominant) ion clusters.[d,e]	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
8	19.8	100	Esters	100	Same as esters, Plant A, Fraction 6.	No masses above 279.
Plant W						
1	20.9	100/100	Aliphatics	100	n-Paraffins.[d,e]	No masses above 424. 1:368(100), 369(45), 353(18)
				100	Same as Plant A, Fraction 2.	
		1	Esters	1	Same as esters, Plant A, Fraction 6.	
2	3.4	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 409. 100:368(100), 369(45), 353(18)
		10	Aromatics	10	Same as Plant A, Fraction 2.	
		100	Esters	100	Same as esters, Plant A, Fraction 6.	
3	13.2	10	Aliphatics	10	Same as Plant A, Fraction 2.	No masses above 485. 100:368(100), 369(45), 353(18)
		1	Esters	1	Same as esters, Plant A, Fraction 6.	
4	8.5	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 495. 10:69(100), 41(80), 43(78) 100:368(100), 369(45), 353(18)
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
5	5.7	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 480. 100:368(100), 369(45), 353(18)
		10	Phenol	10	Same as phenol, Plant A, Fraction 6.	
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
6	4.2	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 451.
		100/10	Phenols	100	Same as Plant F, Fraction 5.	
				10	Same as phenols, Plant A, Fraction 6.	
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
7	14.1	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 495. 10:69(100), 41(80), 43(78) 100:384(100), 383(98), 368(90), 382(68)
		10	Esters	10	Same as esters, Plant A, Fraction 6.	
8	11.7	100	Aliphatics	100	Same as Plant A, Fraction 2.	No masses above 499. 10:Cesium iodide or nathyl iodide. 100:69(100), 41(80), 43(78) 10:383(100), 368(96), 382(72), 369(64)

[a] Relative intensity of the mass-to-charge ratio (intensity relative to dominant ion).

[d] No molecular weight range determination possible.

[e] No composition determination possible.

SECTION 7

BIOASSAY OF SECONDARY EFFLUENTS

The primary objective of the entire wastewater toxicity study is to determine the level of toxicity removal from secondary wastewater achieved by the tertiary treatment technologies selected for the ATMI/EPA BATEA study. To this end, the purpose of this Phase I screening study was to provide chemical and toxicological base-line data on secondary effluents from the 23 textile plants and to select plants for the toxicity removal study (Phase II).

Bioassays used were selected by EPA and included tests for assessment of both health and ecological effects (3). Health effects tests estimated the potential mutagenicity, potential or presumptive carcinogenicity, and potential toxicity of the secondary effluent wastewater samples to mammalian organisms. Ecological effects tests focused on the potential toxicity of samples to vertebrates (fish), invertebrates (daphnids and shrimp), and plants (algae) in freshwater, marine, and terrestrial ecosystems.

Biological testing, as well as chemical and physical parameters, must be considered when assessing the potential impact of industrial or municipal/industrial wastewaters on the aquatic environment. Biological testing involves determination of toxicity for samples of treated effluents. In a toxicity test, aquatic organisms will integrate the synergistic and antagonistic effects of all the effluent components over the duration of exposure.

Although toxicity tests with aquatic organisms can be conducted by applying wastewater samples directly to the test organisms, or by injection or feeding, most tests are conducted by exposing the test organisms to test solutions containing various concentrations of effluent samples. One or more controls are used to provide a measure of test acceptability by giving some indication of test organism health and the suitability of dilution water, test conditions, handling procedures, etc. A control test is an exposure of the organisms to dilution water with no effluent sample added. Bioassay tests are exposures of test organisms to dilution water with effluent samples added. Generally the most important data obtained from a toxicity test are the percentages of test organisms that are affected in a specified way by each concentration of wastewater sample added. The result derived from these data is a measure of the toxicity of the effluent sample to the test organisms under the test conditions.

Acute toxicity tests are used to determine the level of toxic agent that produces an adverse effect on a specified percentage of test organisms in a short period of time. The most common acute toxicity test is the acute mortality test. Experimentally, 50% effect is the most reproducible measure of the toxicity of a toxic agent to a group of test organisms, and 96 hr is often a convenient, reasonably useful exposure duration. The 96-hr median lethal concentration (96-hr LC_{50}) is most often used with fish and macroinvertebrates. Thus the acute mortality test is a statistical estimate of the LC_{50}, which is the concentration of toxicant in dilution water that is lethal to 50% of the test organisms during continuous exposure for a specified period of time. However, the 48-hr median effective concentration (48-hr EC_{50}), based on immobilization, is most often used with daphnids. The terms median lethal concentration (LC_{50}) and median effective concentration (EC_{50}) are consistent with the widely used terms median lethal dose (LD_{50}) and median effective dose (ED_{50}), respectively. "Concentration" refers to the amount of toxicant per unit volume of test solution; "dose" refers to the measured amount of toxicant given to the test organism.

A total of 8 biological systems were used for wastewater toxicity evaluation, utilizing 21 different tester organisms. Specific tests used and the purpose of each bioassay are summarized in Table 26. The tests, testing conditions, and toxicity results for 23 secondary effluent samples are described in this section.

Under guidance of appropriate EPA Technical Advisors, four of the eight bioassays were performed at commercial laboratories experienced with the bioassays. The remaining four bioassays were performed by the EPA Technical Advisor, as shown in Figure 20. Bioassay results were sent to MRC and are included in the following sections.

MICROBIOLOGICAL MUTAGENICITY

Introduction

The purpose of the mutagenicity bioassay was to determine if a chemical mutagen was present in secondary effluents. Nine different bacteria strains and one yeast strain were used in the test because of their individual sensitivities to various classes of chemical compounds. Secondary effluent samples were shipped to Stanford Research Institute (SRI) for mutagenicity testing by *in vitro* microbiological assays with *Salmonella typhimurium* (TA1535, TA1537, TA98, and TA100), *Escherichia coli* WP2, repair-deficient and proficient strains of *Bacillus subtilis* H17 and M45, and *E. coli* Pol A, and the yeast *Saccharomyces cerevisiae*

TABLE 26. BIOASSAY TESTS USED TO EVALUATE THE TOXICITY OF SECONDARY EFFLUENTS

Bioassay test system	Indicator organisms	Purpose of test
Microbial mutagenicity	*Salmonella typhimurium* (Ames test) (Strains TA1535, TA1537, TA98, TA100) *Escherichia coli* (Strains WP2, W3110, p3478) *Bacillus subtilis* (Strains H17 and M45) *Saccharomyces cerevisial* (Strain D3)	To determine if a chemical mutagen (possibly a carcinogen) is present. These microbial strains were selected because of their sensitivity to various classes of chemical compounds.
Cytotoxicity	Rabbit alveolar macrophage (RAM) (viability and ATP determinations) Chinese hamster ovary cells	To measure metabolic impairment and death in mammalian cells. These primary cell cultures have some degree of metabolic repair capability.
Freshwater static bioassay	*Pimephales promelus* (fathead minnow) *Daphnia pulex* (daphnid)	To detect potential toxicity to organisms in aquatic environments.
Freshwater algal assay	*Selenastrum capricornutum*	To detect potential toxicity to aquatic plants.
Marine static bioassay	*Cyprinodon variegatus* (sheepshead minnow) *Palaemonetes pugio* (grass shrimp)	To detect potential toxicity to organisms in a marine environment.
Marine algal assay	*Skeletonema costatum*	To detect potential toxicity to marine plants.
Range finding acute toxicity	Rats (Charles River CD strain)	To detect potential toxicity to whole animals.
Terrestrial ecology	Soil microorganisms	To determine potential effects on soil ecosystems.

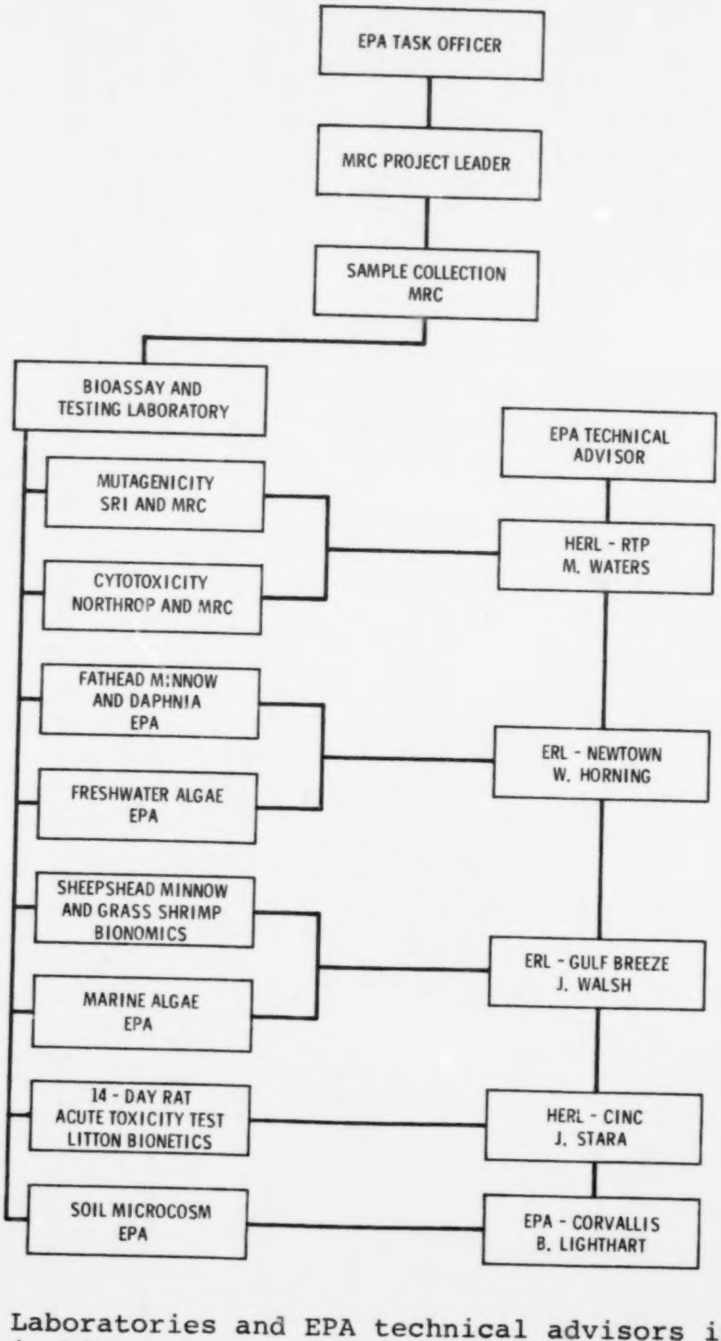
EPA TASK OFFICER
MRC PROJECT LEADER
SAMPLE COLLECTION MRC
BIOASSAY AND TESTING LABORATORY
EPA TECHNICAL ADVISOR
MUTAGENICITY SRI AND MRC
CYTOTOXICITY NORTHROP AND MRC
HERL - RTP M. WATERS
FATHEAD MINNOW AND DAPHNIA EPA
FRESHWATER ALGAE EPA
ERL - NEWTOWN W. HORNING
SHEEPSHEAD MINNOW AND GRASS SHRIMP BIONOMICS
MARINE ALGAE EPA
ERL - GULF BREEZE J. WALSH
14 - DAY RAT ACUTE TOXICITY TEST LITTON BIONETICS
HERL - CINC J. STARA
SOIL MICROCOSM EPA
EPA - CORVALLIS B. LIGHTHART

Figure 20. Laboratories and EPA technical advisors involved in biotesting of effluent samples.

D3. An Aroclor 1254-stimulated, rat-liver homogenate metabolic activation system was included in each procedure.

The assay procedure with *S. typhimurium* has proven to be 85% to 90% accurate in detecting mutagens, and it has about the same accuracy in identifying chemicals that are not carcinogenic (6). The assay procedure with *S. cerevisiae* is about 50% accurate in detecting carcinogens as agents that increase mitotic recombination. The *E. coli* WP2 assay and the microbial sensitivity assay are two additional methods of detecting mutagens. The combination of these assay procedures significantly enhances the probability of detecting potentially hazardous substances.

To date the most sensitive assay for deoxyribonucleic acid (DNA) damage is the induction of mutations in bacteria. The Ames test, the most highly developed of the bacterial mutagenesis tests, used mutant strains of *S. typhimurium* which were specially selected because of their abilities to detect specific types of mutations. For example, the TA1535 strain was designed to detect mutations due to base-pair substitutions. This strain responded particularly well to alkylating agents. Similarly, the TA1537 and TA1538 strains were used to detect frameshift mutations. Tester strains also included mutations which greatly increase their overall sensitivity to mutagens. One of these was responsibly for loss of the DNA excision repair system, while the other was responsible for loss of the lipopolysaccharide barrier that coats the surface of the bacteria, thereby enhancing the penetration of large molecules.

Mutant *Salmonella* tester strains lack the ability to synthesize histidine and are therefore unable to grow unless histidine is supplied. These bacteria are cultured in media containing minimal levels of histidine to sustain growth. Under these conditions only microscopic colonies of bacteria develop during the course of the test. However, if a mutagen is added to the medium, a reversion occurs in a certain number of the bacteria, restoring their ability to synthesize histidine. This reversion (back-mutation) is evidenced by the appearance of visible colonies in the histidine-limited agar, thus indicating the presence of a chemical mutagen.

Many compounds are not directly acting mutagen but are converted to active forms by normal body metabolism. A special microsomal preparation (usually liver) is added in the *Salmonella* tests to simulate *in vivo* metabolic actions. In practice, the substance is tested with and without this microsomal preparation to determine whether it requires metabolic transformation or is, itself, mutagenically active.

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(6) McCann, J., E. Choi, E. Yamasaki, and B. N. Ames. Detection of Carcinogens as Mutagens in the *Salmonella*/Microsome Test: Assay of 300 Chemicals. Proceedings of the National Academy of Science, 72:5135-5139, 1975.

The testing procedure used for each type of microbe and the bioassay results of each test are described below.

Bioassay Procedures

Each secondary effluent sample was shipped and stored in the laboratory at 4°C. Preliminary experiments on the first two samples received indicated microbial contamination when an aliquot of the sample was incubated on a culture medium. Therefore, each sample was filtered before it was tested in any microbial system. Nalgene filters (0.45 μm) were used. Approximately 50 x 10^{-6} m^3 of each sample was filtered; the remaining 200 x 10^{-6} m^3 was stored for possible future testing.

Four strains of *S. typhimurium* (TA1535, TA1537, TA98, and TA100) were obtained from Dr. Bruce Ames of the University of California at Berkeley and stored in 10% sterile glycerol at -80°C. New stock cultures were prepared every two months from single colony reisolates that were checked for their genotypic characteristics and for presence of the plasmid. For each experiment, an inoculum from the stock cultures was grown overnight at 37°C in nutrient broth (Oxoid, CM67). After stationary overnight growth, the cultures were shaken for 3 hr to 4 hr to ensure optimal growth.

The metabolic activation mixture used for each experiment consisted of

- 1.0 x 10^{-6} m^3 of S-9 rat liver fraction
- 0.2 x 10^{-6} m^3 of magnesium chloride (0.4 M) and potassium chloride (1.65 M)
- 0.05 x 10^{-6} m^3 of glucose-6-phosphate (1 M)
- 0.4 x 10^{-6} m^3 of nicotine adenine dinucleotide phosphate
- 5.0 x 10^{-6} m^3 of sodium phosphate (0.2 M, pH 7.4)
- 3.35 x 10^{-6} m^3 of water

For each experiment, the following solutions, listed in the order of addition, were added to a sterile 13 mm x 100 mm test tube placed in a 43°C heating block:

- 2.00 x 10^{-6} m^3 of 0.6% agar (containing 0.05 mM histidine and 0.05 mM biotin)
- 0.05 x 10^{-6} m^3 of indicator organisms
- 0.50 x 10^{-6} m^3 of metabolic activation mixture
- 0.05 x 10^{-6} m^3 of the secondary effluent sample

Dimethylsulfoxide (DMSO) was added to each sample to improve the water solubility of organic compounds. The resulting mixture was gently stirred and poured onto minimal agar plates. These plates consisted of 15 kg of agar, 50 kg of glucose, 0.2 kg of magnesium sulfate ($MgSO_4 \cdot 7\ H_2O$), 2 kg of citric acid monohydrate, 10 kg of potassium orthophosphite, and 3.5 kg of sodium ammonium phosphate, per cubic meter. After the top agar had set, the plates were incubated at 37°C for 2 days. Then the number of revertant colonies was counted. Each sample was run in duplicate.

All samples were run with both positive and negative controls, with and without metabolic activation at five concentrations. Positive controls were run using various combinations of 2-anthramine, 9-aminoacridine, β-propiolacetone, sodium azide, n-methyl-n'-nitro-n-nitrosoguanidine, daunomycin, and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF2). Controls were also run on DMSO.

The *S. cerevisiae* tester strain was stored at -80°C. For each experiment, the tester strain was inoculated in 1% tryptone and 0.5% yeast extract and grown overnight at 37°C with aeration.

The *in vitro* yeast mitotic recombination assay in suspension was conducted as follows. The overnight culture was centrifuged, and cells were resuspended at a concentration of 10^8 cells per 10^{-6} m^3 in a 67-mM phosphate buffer (pH 7.4). The following solutions were added to each sterile test tube:

- 1.30×10^{-6} m^3 of *S. cerevisiae*
- 0.50×10^{-6} m^3 of either the metabolic activation mixture or buffer
- 0.20×10^{-6} m^3 of the secondary effluent sample

Because many organic chemicals are not appreciably water soluble, DMSO was added as the solvent for the secondary effluent sample. Several doses of the chemical [up to 5%, weight-to-volume ratio (w/v) or volume-to-volume ratio (v/v)] were tested in each experiment, and appropriate controls were included.

The suspension mixture was incubated at 30°C for 4 hr on a roller drum. The sample was diluted serially in a sterile physiological saline, and volumes of 0.2×10^{-6} m^3 of the 10^{-5} and 10^{-3} dilutions were spread on tryptone-yeast agar plates; five plates were used for the 10^{-3} dilution and three plates for the 10^{-5} dilution. These plates were incubated for 2 days at 30°C, followed by 2 days at 4°C to enhance the development of the red pigment. Plates of the 10^{-3} dilution were scanned with a dissection microscope at 10 X magnification, and the number of red colonies or red sectors (mitotic recombinants) was recorded. The surviving fraction of organisms was determined from the number of colonies appearing on the plates of the 10^{-5} dilution.

The number of mitotic recombinants was calculated per 10^5 survivors. A positive response in this assay was indicated by a dose-related increase of more than threefold in the absolute number of mitotic recombinants per 10^{-6} m^3 as well as in the relative number of mitotic recombinants per 10^5 survivors. Positive, negative, and reagent controls were run at four concentrations with each test, with and without metabolic activation. Positive controls were performed using 1,2,3,4-diepoxybutane.

A procedure similar to the Ames *Salmonella* assay was used to measure the reversion of *E. coli* WP2 to tryptophan independence. However, the minimal agar was supplemented with 1.25 g of Oxoid nutrient broth (CM67) per 10^{-3} m^3 to provide each plate with the trace of tryptophan required for enhancement of any mutagenic effect of the test chemical. No additional tryptophan was added to the top agar. The positive controls used for the Ames test were also used for the *E. coli* WP2 test.

As an alternative to reversion of the mutated tryptophan gene, WP2 may undergo a forward suppressor mutation in a tryptophan transfer ribonucleic acid (RNA) gene to obtain tryptophan independence. The test did not distinguish experimentally between true revertants and suppressor mutants (although the latter tend to form smaller colonies).

E. coli strains W3110 and p3478 and *B. subtilis* strains H17 and M45 were stored in the laboratory at -80°C. Inoculums from the frozen stocks were grown overnight at 37°C with shaking in a nutrient broth. The broth contained 1% of tryptone and 0.5% yeast extract, and was supplemented with 5 µg of thymine per 10^{-6} m^3. To 2 x 10^{-6} m^3 of top agar containing 0.6% agar was added 0.1 x 10^{-6} m^3 of the test culture. This suspension was mixed and poured onto plates containing nutrient broth and 2% agar.

After the soft agar solidified, a sterile filter disc impregnated with a secondary effluent sample was placed in the center of the plate. Plates were incubated at 37°C for 16 hr, and the width of the zone of toxicity or inhibition of growth was measured. Several concentrations of chemical were tested to accurately detect differences in the zones of growth inhibition, because higher initial concentrations lead to steep concentration gradients that may reduce the difference in growth inhibition of the test strains.

The positive control for this assay was 2 mg of 1-phenyl-3,3-dimethyltriazene placed on the disc. Larger zones of inhibition were observed in the DNA repair-deficient strains (p3478 and M45). The negative control was 20 µg of chloramphenicol. Equal zones of inhibition were observed in all four strains since the toxicity of chloramphenicol did not depend on a mechanism that leads to DNA damage.

Results

SRI tested secondary effluent wastewater samples from 22 of the 23 basic textile plants for bacterial mutagenicity using 10 tester microorganisms and 3 different assay systems. The secondary effluent sample from Plant E was lost in shipment.

The voluminous amount of mutagenicity raw data generated by SRI are given in Reference 7 and a summary of the results is given in the following paragraphs.

All 22 of the samples were tested twice in the standard Ames *Salmonella* microsome procedure using four test strains: TA1535, TA1537, TA98, and TA100. A metabolic activation mixture was included in each experiment. Each sample was tested to a maximum dose of 1×10^{-6} m^3 of sample per plate (the maximum amount possible in 2×10^{-6} m^3 of top agar). The second experiment was a confirming experiment. None of the samples caused an increase in the number of histidine-independent revertants above the normal background, thus no chemical mutagen was detected.

Twenty-two samples were tested in the *E. coli* WP2 strain. None of the samples caused an increase in the number of tryptophan-independent revertants above the normal background. Twenty-two samples were tested in the *S. cerevisiae* D3 suspension assay, with and without a metabolic activation system. The maximum concentration tested was 50% v/v. None of these samples caused an increase in the number of mitotic recombinants above the normal background.

The samples were also tested in the microbial inhibition assay using DNA repair-deficient and -proficient strains of *E. coli* and *B. subtilis*. The maximum dose tested was 20×10^{-9} m^3. Each sample was applied to a filter disc on the plate. None of the samples was toxic to any of the strains of the organisms used.

All 22 secondary effluent samples were tested in three assay systems. In each assay, the maximum possible dose was tested. None of the samples gave a mutagenic or toxic response in any strain in any assay or experiment.

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(7) Poole, D. C. and V. F. Simmon. Final Report of *in Vitro* Microbiological Studies of Twenty-two Wastewater Effluent Samples. Contract 68-01-2458, U.S. Environmental Protection Agency, Biomedical Research Branch, Research Triangle Park, North Carolina, November 1977. 111 pp.

MRC Ames Testing

To provide backup duplicate results to the Ames tests performed by SRI, MRC performed the Ames test on eight randomly selected secondary effluent samples from Plants D, H, J, M, P, R, Y, and Z.

Biotest procedures used by MRC were the same as those used by SRI. Final test results indicated no positive responses on filtered, unconcentrated effluent samples. Samples from Plants D, P, and R were rerun on selected strains with no indication of a positive response.

CYTOTOXICITY ASSAY

Introduction

Cytotoxicity (cell toxicity) assays were performed to measure quantitatively any cellular metabolic impairment and death resulting from exposure *in vitro* to secondary effluent samples. Primary cell cultures, such as the rabbit alveolar macrophage (RAM) used in this study, exhibit many of the metabolic and functional attributes of the *in vitro* state. These cells can therefore combat, to some degree, the effects of chemical mutagens on mammalian cells.

Recently this system has been applied in evaluating the relative cellular toxicity of hazardous metallic salts and industrial air particulates (8). As compared to conventional whole animal tests for acute toxicity, these cytotoxicity assays are more rapid, less costly, and require less sample. These tests provide useful information about the relative toxicity of unknown samples. However, it should be understood that because the assays employ isolated cells and not intact animals, they can provide only preliminary information about the ultimate human health hazards of toxic chemicals.

Two tests were used to measure the toxic effects of secondary effluent samples on rabbit alvaolar macrophage: viability and adenosine triphosphate (ATP) production. Viability refers to the ability of cells to survive, and it was measured by the trypan blue dye exclusion method. Living (viable) cells do not absorb trypan blue dye. Therefore, the measure of cell mortality is the number of blue (dead) cells counted after exposure to the sample.

Adenosine triphosphate (ATP) is a coenzyme in mammalian cells that plays an important role in energy metabolism. Living cells synthesize ATP. Therefore, another measure of cell mortality or inhibition was the quantity of ATP produced by a cell culture exposed to the secondary effluent sample compared to the amount produced by an identical culture not exposed to the solution.

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(8) Waters, M. D., D. E. Gardner, C. Aranyi, and D. L. Coffin. Metal Toxicity of Rabbit Alveolar Macrophages *in Vitro*. Environmental Research, 9(1):32-47, 1975.

Both of these methods were used to evaluate secondary effluent toxicity to RAM. The measure of toxicity was expressed as EC_{20} or EC_{50}; i.e., the concentration of secondary effluent that inhibits RAM metabolism by 20% or 50% over a specified time period (20 hr).

The following section discusses the test procedure used to evaluate secondary effluent toxicity and the bioassay results.

Bioassay Procedure

Rabbit alveolar macrophage primary cell cultures were obtained from New Zealand white rabbits of both sexes. Each of the 23 secondary effluent samples was filtered through a 0.45-μm filter. There was no concentrating of the samples prior to testing. Each sample was prepared in five dilutions: 6, 20, 60, 200, and 600 x 10^{-6} m^3 sample per 10^{-3} m^3 solution. To each concentration, fetal calf serum (heat inactivated) was added to give a final serum concentration of 10%. Antibiotics were added to give 100 units per 10^{-6} m^3 penicillin, and 100 μg combined streptomycin and kanamycin per 10^{-6} m^3. The pH of each concentration was recorded, but no adjustments were made.

Samples were then added to Falcon cluster dishes, 1.5 x 10^{-6} m^3 per well. A volume of 0.5 x 10^{-6} m^3 of complete 1X Medium 199 (with 10% fetal calf serum and antibiotics) containing approximately 2 x 10^6 rabbit alveolar macrophages per 10^{-6} m^3 was added to each well and gently mixed. Dishes were then incubated for 20 hr at 37°C in a humidified atmosphere of 5% CO_2 in air on a Bellco rocker platform.

At the end of the 20-hr exposure, the medium containing unattached cells was removed from each well and transferred to a separate test tube. A volume of 10^{-6} m^3 of 0.25% trypsin was added to each well and left until the cells were removed from the dish. This was then combined with the original pouroff and mixed to inactivate the trypsin.

Cell counts, viabilities, and ATP determinations were then performed.

Viability was determined by trypan blue dye exclusion. Filtered samples were counted in the Cytograf; unfiltered samples, because of the particulate matter present, were counted in a hemocytometer. For hemocytometer counts, 1 part of 0.4% trypan blue was added to 5 parts cell suspension and counted after a 5-min exposure. For Cytograf counts, dilutions, usually fourfold, were made with cold

0.85% saline to yield a suspension of no more than 2 x 10^5 cells/10^{-6} m^3. Trypan blue was diluted (immediately before use) with 0.85% saline to a final concentration of 0.01% and added to an equal volume of cell suspension. Simultaneous determinations of cell viability and cell numbers per 10^{-6} m^3 of cell suspension were made. The numbers of viable cells were expressed as a percentage of the number of cells in control cultures; viability was expressed as the concentration of secondary effluent which inhibited 20% and 50% of the test cells (EC_{20} and EC_{50}).

ATP was determined according to a procedure supplied with the Du Pont Model 760 Luminescence Biometer. Dimethyl sulfoxide (0.4 x 10^{-6} m^3) was used to extract ATP from a 0.1 x 10^{-6} m^3 aliquot of trypsinized cell suspension containing 0.3 to 0.4 x 10^5 cells. After 2 min at room temperature, 2.5 x 10^{-6} m^3 of cold 0.1 M morpholinopropane sulfonic acid (MOPS) at pH 7.4 was added to buffer the extracted sample. The tube containing the buffered sample was then placed in an ice bath. Aliquots of 10^{-8} m^3 were injected into the luminescence meter's reaction cuvette containing 0.7 mM luciferin (crystalline), 100 units luciferase (purified and stabilized), and 0.01 M magnesium sulfate in a total volume of 10^{-7} m^3 of 0.01 M MOPS buffer, pH 7.4 at 25°C. Light emitted from the reaction cuvette was measured photometrically in the luminescence meter and was proportional to the ATP concentration of the sample. ATP values were expressed per 10^6 cells and as a percent of the control cells.

Nutrient agar plates were streaked with 0.5 x 10^{-6} m^3 from each sample (unfiltered) and antibiotic sensitivity discs were added for a 24-hr incubation period. This was done to ascertain what antibiotics were capable of suppressing growth of any bacteria present in the samples. Antibiotics present in the culture medium were found to be capable of inhibiting bacterial growth so that the samples could be tested unfiltered.

Results

All determinations were performed in duplicate or triplicate. The voluminous amount of cytotoxicity raw data generated by Northrop are given in Reference 9 and a summary of the results are given below.

Because cell viability could be considered a binomial response, the arc-sine transformation was employed in the regression

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(9) Campbell, J. A., H. F. Stack, and P. R. Williams. Cytotoxicity Screening of Twenty-three Textile Mill Effluent Water Samples Utilizing the Rabbit Alveolar Macrophage Assay. Contract 68-02-2566, U.S. Environmental Protection Agency, Biomedical Research Branch, Research Triangle Park, North Carolina, December 1977. 86 pp.

analysis. Samples listed as nondeterminable were such high extrapolations that they could not be considered significant estimates.

Table 27 shows the estimated EC_{20} and EC_{50} concentrations for the filtered effluents. The EC values are the concentrations expected to cause a decrease in viability and ATP by 20% and 50%, respectively. Control values were routinely 92% to 100%.

TABLE 27. ESTIMATED EC_{20} AND EC_{50} VALUES FOR CYTOTOXICITY SCREENING OF FILTERED SECONDARY EFFLUENT SAMPLES

	Percent effluent					Percent effluent			
	Viability		ATP[a]			Viability		ATP[a]	
Plant	EC_{20}	EC_{50}	EC_{20}	EC_{50}	Plant	EC_{20}	EC_{50}	EC_{20}	EC_{50}
A					M				
B					N	13.3		3.8	12.8
C[b]	16.8		6.1	33.5	P				
C[c]			–[d]	–[d]	R				
D					S	–[d]	–[d]	–[d]	–[d]
E					T			2.5	
F			9.4		U				
G					V				
H					W			13.7	
J					X			4.8	
K					Y				
L			4.0	35.1	Z				

Note.—Blanks indicate data not determinable.

[a] Adenosine triphosphate. [b] pH equals 9.1 not adjusted before testing.

[c] pH adjusted to 7.2. [d] Test not performed.

Samples from Plant N and C caused the greatest response by viability and ATP determinations. The response of Sample C was largely due to its high (9.1) pH. When testing was repeated with the pH adjusted to 7.2, there was much less response.

Because the antibiotic sensitivity testing showed that samples could be tested without prior filtration, five samples were retested without prior filtration. Much of the solid material removed by filtration appeared to be biological material (e.g., algae) by microscopic observation. In each instance, the unfiltered sample caused greater response than the filtered one.

MRC Clonal Assay

MRC performed clonal assay acute toxicity tests using Chinese hamster ovary cells (CHO-K1) on selected secondary effluent samples. The purpose of these tests was to evaluate the response of another test system to complex environmental samples. MRC has developed this *in vitro* clonal assay for measuring acute toxicity of compounds using CHO-Kl cells. This test is a modification of a clonal assay described by Malcolm (10). Preliminary studies indicate that the sensitivity of the CHO-Kl clonal assay appears to be two orders of magnitude greater than that of the WI-38 assay.

Secondary effluent samples from eight textile plants (Plants D, H, J, M, P, R, Y, and Z) were selected at random and analyzed according to the following procedure.

Test Procedure--
Effluent samples for this test were filtered through 0.45-μm and 0.22-μm filters. Samples were run at 5 to 7 concentration levels using approximately 300 to 500 cells that had been plated on the previous day. After incubation at 37°C for 6 days to 7 days, the media and sample were removed and the cells were fixed, stained, and counted. Results are reported as experimental versus controls or percent survival. A detailed test procedure is given in Table 28.

Results--
Screening tests were first performed on the eight samples to determine whether any of them were toxic and in what concentration range. Results showed that secondary effluent samples from Plants P, R, Y, and Z had no acute toxicity to the CHO-Kl cells, but samples from Plants D, H, J, and M did exhibit toxicity. The latter four samples were therefore rerun using narrower sample concentrations.

Test results are presented in Figure 21. The range bar associated with each data point corresponds to the standard deviation of that value.

Graphical interpolation of Figure 21 yields the following LC_{50} values (in percent of secondary effluent sample) for Plants D, H, J, and M: 2.4%, 13.3%, 18.5%, and 3.0%, respectively. These values indicate that the secondary effluent from Plants D and M contain chemical species more acutely toxic to CHO-Kl cells than do samples from Plants H and J.

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(10) Malcolm, A. R., B. H. Pringle, and H. W. Fisher. Chemical Toxicity Studies with Cultured Mammalian Cells. In: Bioassay Techniques and Environmental Chemistry, G. E. Glass, ed. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, 1974. pp. 217-230.

TABLE 28. CHO-K1 CLONAL CYTOTOXICITY TEST

Cell line:	Chinese hamster ovary epithelial cells ATCC No. CCL 61
Medium:	F-12 GIBCO No. H-17 10.8 x 10^3 g/m^3 Sodium hydrogen carbonate 10% Fetal calf serum, virus, mycoplasma screened GIBCO No. 629
Incubation:	37°C, 5% CO_2, Saturated humidity
Samples:	6 Controls (blank) 5 to 7 Concentrations of test compound in triplicate 5 Concentrations of a positive toxic control in triplicate

Test procedure
To stock CHO-K1, add 5 x 10^{-6} m^3 0.25% trypsin at 37°C for 5 min.
Shake cells and add to centrifuge tube.
Add 5 x 10^{-6} m^3 media to flask, shake, and add to centrifuge tube.
Centrifuge 5 min at 1,200 g, pour off liquid, retaining cells.
Add 10 x 10^{-6} m^3 medium, shake, centrifuge 5 min, pour off medium.
Add 10 x 10^{-6} m^3 medium, shake.
Make hemocytometer count of trypsinized cells.
Dilute so that 5 x 10^{-6} m^3 media contain 300 to 500 cells.
Add 5 x 10^{-6} m^3 media and cells to T-25 flasks.
Incubate 12 hr to 18 hr to allow attachment using normal media.
Replace 5 x 10^{-6} m^3 of media and sample.
Incubate 6 days to 7 days total.
Fix with 10% formaldehyde/0.5% sodium chloride/4% methanol for 30 min.
Stain with crystal violet (0.04% for 15 min).
Count clonal colonies of remaining cells macroscopically using Fisher Count-All Model 600.
Score with respect to experimental vs. controls as percent survival.

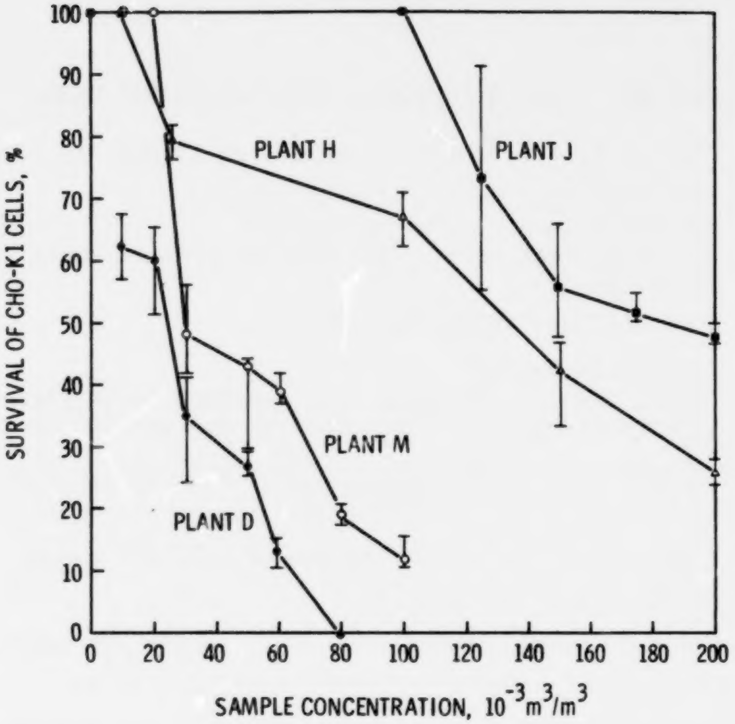

Figure 21. Results of CHO-K1 clonal assay.

FRESHWATER ECOLOGY TOXICITY

Algal Assay Bottle Test

Algal assay was performed to estimate the potential toxicity of secondary effluents on aquatic plants. The algal assay was based on the principle that growth is limited by the essential nutrients that are in shortest supply. The test was designed to quantify the biological response (algal growth) to changes in concentrations of nutrients, and to determine whether various effluents were stimulatory or inhibitory to algae. These measurements were made by adding a selected test alga to the effluent and determining its growth response.

The freshwater algae testing series was performed at the Environmental Research Laboratory (ERL, Corvallis, Oregon) under the direction of W. E. Miller. Each effluent sample (0.01 m^3 from each of the 23 plants) as apportioned in three autoclavable, 0.004-m^3 plastic bottles, was packed in ice and flown air freight to Corvallis, Oregon. Most samples arrived at the laboratory within 36 hr from the time they were shipped. The following paragraphs summarize the sample handling and analysis procedures used at ERL. An EPA manual (3) gives a detailed description of the procedure.

Each 0.01-m^3 textile effluent sample was thoroughly composited in the laboratory in a 0.02-m^3 cubitainer, then redistributed into bottles. A 0.001-m^3 aliquot was taken for soil microcosm studies. Samples were stored in the dark at 5°C.

Well water from the Western Fish Toxicology Station was used for dilution water and for control samples. The water was filtered with a 0.45-μm porosity membrane filter and pad to remove any particulate matter before mixing with raw effluent. Dilutions, Table 29, were made in a 0.02-m^3 cubitainer for a total of 0.016 m^3 of diluted effluent.

TABLE 29. DILUTIONS USED FOR FRESHWATER ALGAL TESTS

Dilution, %	Effluent volume, x 10^{-4} m^3	Well water volume, x 10^{-3} m^3
2	3.2	15.68
5	8.0	15.20
10	16.0	14.40
20	32.0	12.80
Total volume used	59.2	58.08

Each dilution of 0.016 m^3 was divided into four parts of 0.004 m^3 each for four different treatments: One part was autoclaved and filtered, one was autoclaved only, one was filtered and autoclaved, and one was filtered only.

Diluted samples were autoclaved in polypropylene bottles (washed with 10% hydrochloric acid and autoclaved before use) at a pressure of 108 kPa at 10 min/10^{-3} m^3. Diluted samples were filtered with a 0.45-μm porosity membrane filter and pad. During and after treatment, diluted samples were stored in the dark at 5°C.

The pH values were taken on raw effluent and on diluted effluent before and after treatment to determine changes in pH that can affect growth. Each secondary effluent sample was analyzed by MRC for the following nutrient indicators: *o*-phosphate, ammonia, nitrite, nitrate, total Kjeldahl nitrogen, total phosphorus, and total organic carbon, Table 30 (4, 5).

The algal assay bottle test procedure used *Selenastrum capricornutum* Printz as the test alga and was used to assess algal growth response to 23 secondary effluent wastewater samples. Growth response was measured as net biomass produced (gram dry weight per cubic meter of sample), i.e., the total biomass in the sample minus the biomass produced in a control sample using well water. Algal response was expressed as percent stimulation or inhibition at the 20% wastewater concentration as compared to the control sample. Tests were performed on two types of

TABLE 30. NUTRIENT ANALYSIS OF SECONDARY EFFLUENT SAMPLES

Plant code	Nutrient, g/m³						
	Nitrite	Nitrate	Ammonia	Total Kjehldahl nitrogen	*o*-Phosphate	Total phosphorus	Total organic carbon
A	0.06	1.9	12.8	21.3	1.0	0.39	
B	0	0.002	2.5	4.2	7.3	6.0	29.0
C	4.64	23.3	3.4	1.85	1.08	0.50	219
D	7.5	0.08	0.20	4.5	1.6	2.48	36
E	<0.02	40	1.13	6.75	1.11	1.19	31.8
F	0.04	0	1.54	14.8	0.56	9.7	57.5
G	<0.02	1.3	0.40	12	5.02	6.29	
H	16.8	0.69	0.02	5.5	0.06	0.39	84
J	11.9	0.24	1.25	2.3	0.28	1.68	150
K	<0.02	0.16	0.40	5.25	0.82	1.05	19.1
L	0.86	13.5	0.5	1.9	0.88	1.98	42.9
M	0.27	0.30	0.84	6.15	0.55	3.03	84
N	<0.02	5.5	12.8	17.0	11.2	2.05	90.4
P	7.3	0.08	0.20	2.9	0.02	3.12	54
R	1.82	0.015	2.6	19.8	0.06	1.82	244
S	<0.02	0.23	1.65	40	3.1	5.23	261
T	0.04	0.80	13.6	30.2	6.4	14.9	142
U	<0.02	0.8	5.44	11.3	2.96	2.43	
V	0.07	1.0	1.26	6.3	0.11	0.76	
W	0.145	12.3	0.38	7.4	0.075	0.50	199
X	0.44	0.033	0.65	5.25	0	4.87	94
Y	8.5	0.05	1.9	5.75	9.9	14.3	41.8
Z	<0.02	0.94	0.85	4.75	0.40	0.54	40

Note.—Blanks indicate analysis not performed.

pretreated samples: 1) filtered followed by autoclaving, and 2) filtered only. Results are given in Table 31.

TABLE 31. RESULTS OF FRESHWATER ALGAE BIOASSAY TESTS

Plant	14-Day growth response in 20% secondary effluent as compared to control well water			
	Filtered only		Filtered-autoclaved	
	Percent inhibition	Percent stimulation	Percent inhibition	Percent stimulation
A	53	0	33	0
B	0	83	0	44
C	0	187	0	146
D	0	100	0	125
E	95[a]	0	95[a]	0
F	0	598	0	866
G	0	390	0	578
H	92	0	95	0
J	0	76	0	217
K	0	57	0	243
L	81	0	0	98
M	0	149	0	291
N	95[a]	0	95[a]	0
P	0	38	5	0
R	95[b]	0	93[b]	0
S	0	382	0	365
T	0	1,911	0	2,362
U	0	377	0	639
V	0	232	0	503
W	95	0	95	0
X	0	163	0	348
Y	0	261	0	365
Z	84	0	17	0

[a] 95% growth inhibition in 2% solution of secondary effluent.

[b] Sample inadvertently collected prior to the settling pond.

Five distinct growth patterns were discerned in the initial screening; they are described in the following sample subsets.

Two samples (Plants E and N) failed to support growth of the test alga in all waste concentration levels (2%, 5%, 10%, and 20%). This response has been attributed to toxicity since nitrogen (N) and phosphorus (P) concentrations (2% level) were adequate to support up to 8.0 g dry wt/m^3 of *S. capricornutum*.

Seven samples (Plants B, D, J, K, P, X, and Z) supported similar growth at all dilutions ranging from 5.5 to 16.0 g dry wt/m^3 of *S. capricornutum*, depending on nutrient bioavailability within waste samples. Failure of the growth response to increase at a rate proportional to the analyzed incremental concentration of nitrogen and phosphorus in these samples suggests that some constituent other than nitrogen and phosphorus is limiting the maximum yield. However, the suboptimal yield obtained is considered to be stimulatory for the support of test alga.

Four samples (Plants H, L, R, and W) supported growth at the 2% and 5% levels but inhibited growth at the 10% and 20% concentrations.

Seven samples (Plants C, F, G, M, S, V, and Y) supported increasing growth of test alga with similar increase in waste concentration, proportional to the bioavailable (but not chemically analyzed) inorganic nitrogen content. The growth thus obtained indicates that these wastes are highly stimulatory for the support of test alga.

Two samples (Plants T and U) were extremely stimulatory, 124.5 and 29.1 g dry wt/m^3, respectively, for these wastes at the 20% concentration level. Growth obtained in these samples was directly proportional to their chemical nitrogen and phosphorus content, indicating its complete availability for support of algal growth.

Using test results from the filtered-and-autoclaved and filtered-only samples, ERL was able to rank the secondary effluent samples in terms of inhibition and stimulation; results are shown in Table 32. In those samples categorized as nontoxic, the primary limiting factor regulating growth response of the test alga was bioavailability and utilization of the total soluble inorganic nitrogen (TSIN equals nitrite, nitrate, and ammonia). Shiroyama, Miller, and Greene (11) demonstrated that maximum yield for *Selenastrum capricornutum* Printz is predictable provided the TSIN is known, other essential nutrients are in adequate supply, and toxicants are absent. Under these conditions 0.001 g/m^3 of TSIN can yield 0.038 g/m^3 dry weight of the alga. Based on this

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(11) Shiroyama, T., W. E. Miller, and J. C. Greene. The Efforts of Nitrogen and Phosphorus on the Growth of *Selenastrum Capricornutum* Printz. EPA-606/3-75-034, U.S. Environmental Protection Agency, Corvallis, Oregon, March 1975. pp. 132-142.

TABLE 32. RELATIVE RANKING OF TEXTILE PLANTS BY TOXIC AND STIMULATORY EFFECTS OF SECONDARY WASTEWATER ON *S. CAPRICORNUTUM*

Filtered and autoclaved samples			Filtered only samples		
Toxic rating	Plant rank	Stimulatory rating	Toxic rating	Plant rank	Stimulatory rating
Most toxic	E, N	Nonstimulatory	Most toxic	E, N	Nonstimulatory
"	W, H	"	"	W	"
Least toxic	R,[a] P, L, B, Z, A	"	"	R,[a] H	"
Nontoxic	D	Least stimulatory	Least toxic	A, Z, L	"
"	C	"	Nontoxic	P	Least stimulatory
"	Y	"	"	J	"
"	K	"	"	B	"
"	J	"	"	K	"
"	M	"	"	M	"
"	X	"	"	D	"
"	S	"	"	X	"
"	F	"	"	Y	"
"	V	"	"	C	"
"	G	"	"	U	"
"	U	"	"	G	"
"	T	Most stimulatory	"	F	"
			"	V	"
			"	T	Most stimulatory

[a] Sample inadvertently collected prior to the setting pond.

information a linear regression between biomass produced in the textile waste samples and that predicted from the TSIN content of the test water showed a correlation coefficient in the filtered followed by autoclaving and filtered only samples of 0.980 and 0.989, respectively, resulting in dry weight yield per unit concentration of TSIN relationship of 0.910 and 0.997, respectively. These relationships indicate that the complexity of the textile water samples under the two pretreated conditions does not affect the algal from utilizing the essential nutrients in obtaining its maximum yield.

Acute Static Bioassays with Freshwater Fish and *Daphnia*

The acute static bioassay technique with freshwater animals provided an easy measure of toxicity and was recommended by EPA for the wastewater assessment (3). Fathead minnow (*Pimephales promelus*) and *Daphnia pulex* were the selected test animals because they are a readily available, hardy species, and they can be conveniently and economically maintained in a laboratory (3).

Primary objections to the following procedure are that the recommended dilution water may not closely simulate receiving water characteristics, and the fathead minnow may not be representative of the most sensitive species in a given geographical area. However, the procedure does adequately serve to develop relative toxicity data for the purpose of ranking industries based on the toxicity of their effluents.

This series of tests was performed at the EPA Fish Toxicology Station (Newtown, Ohio) under the direction of Mr. W. Horning. Each of the three 0.02-m^3 glass bottles of effluent from 23 plants was packed in ice and shipped by air freight.

The fathead minnow test utilized 16 x 10^{-3} m^3, wide mouthed jars. A control dilution and effluent dilutions of 100%, 60%, 36%, 21.6%, 13%, 7.8%, and 4.7% effluent were set up. A total volume of 0.015 m^3 was used in each test jar. Ten fish were randomly distributed to each jar. Duration of the test was 96 hr, and temperature ranged from 20.5°C to 21.6°C. Aeration was not used during the test. At the end of the test, the fish length and weight were determined. Length ranged from 28 mm to 44 mm, with an average length of 33 mm. Weight ranged from 0.18 g to 0.80 g, with an average weight of 0.29 g.

Dissolved oxygen, pH, specific conductivity, temperature, and turbidity were determined in each test jar at the beginning of the test and every 24 hr through the end of the test. At the end of each 24-hr period, the number of fish surviving was recorded, and dead fish were removed.

The same test procedures and dilutions of effluent used for the fathead minnow test with *D. pulex*. The *D. pulex* were from a laboratory culture maintained at the Newtown Fish Toxicology Station. Estimated LC$_{50}$ and EC$_{50}$ values and 95% confidence limits were reported where possible and are shown in Table 33. Data were evaluated with Probit Analysis whenever possible, with Moving Average Angle where Probit was not applicable.

TABLE 33. ACUTE TOXICITY DATA FOR FATHEAD MINNOW AND *DAPHNIA PULEX*

Plant code	Fathead minnow, percent effluent concentration: 96-Hr LC$_{50}$	Fathead minnow, percent effluent concentration: 95% Confidence limit	Statistical analysis	*Daphnia pulex*, percent effluent concentration: 48-Hr EC$_{50}$	*Daphnia pulex*, percent effluent concentration: 95% Confidence limit	Statistical analysis
A	19.0	15.5 to 24.0	MA[a]	9.0	6.8 to 11.6	P[b]
B	NAT[c]			NAT		
C	46.5	37.6 to 57.5	MA	41.0	32.4 to 50.2	MA
D	NAT			NAT		
E	NAT			7.8	5.6 to 9.8	P
F	NAT			81.7	66.7 to 101.6	P
G	64.7	57.0 to 74.8	MA	62.4	54.3 to 72.7	MA
H	–[d]			–[e]		
J	NAT			NAT		
K	NAT			NAT		
L	23.5	18.3 to 28.7	MA	28.0		MA
M	NAT			60.0	40.7 to 89.0	MA
N	48.8	38.8 to 61.8	P	–[f]		
P	NAT			NAT		
R[g]	16.5	12.4 to 21.7	P	8.0	6.1 to 8.0	MA
S	NAT			NSA[h]		
T	46.5	37.6 to 57.5	MA	NAT		
U	NAT			12.1	8.7 to 16.3	P
V	36.0	27.4 to 43.9	MA	9.4	7.1 to 12.2	P
W	55.2	45.2 to 70.7	MA	6.3	3.7 to 8.4	P
X	NAT			NAT		
Y	NAT			NAT		
Z	NAT			42.6	30.8 to 64.1	P

[a] Moving average angle. [b] Probit. [c] No acute toxicity. [d] A bad batch of fish nullified this test.

[e] 40% Dead at 100% concentration. [f] 100% Dead at all dilutions.

[g] Sample inadvertently collected prior to the settling pond.

[h] No statistical analysis; heavy solids concentration obscured the analysis; the sample did not appear to be acutely toxic.

Generally, the toxicity of textile mill effluent samples was exerted within the first 24 hr for both the fathead minnow and *D. pulex*. The relative sensitivity of the animals cannot be differentiated on the basis of these samples. In 5 out of the 15 samples, *D. pulex* appeared to be somewhat more sensitive than the fathead minnow. In one instance, the effluent was not acutely toxic to *D. pulex* but was acutely toxic to fathead minnow, with an estimated LC_{50} value of 46.5% effluent dilution. It should be noted that 8 samples were acutely toxic to fathead minnow and 10 were acutely toxic to *D. pulex*. Thus, it is desirable to use, when feasible, more than 1 organism to evaluate the toxicity of an effluent.

Reactions of test organisms to each effluent sample are briefly described in Appendix D.

MARINE ECOLOGY TOXICITY

Bioassay with Unicellular Marine Algae

Unicellular algae are important constituents of marine ecosystems. They are comprised of a variety of species that have different growth rates, photosynthetic rates, nutrient requirements, and other functions that regulate species composition and diversity in the community in relation to environmental parameters. The algal community, through photosynthesis, produces most of the food and oxygen used in the marine ecosystem.

It is well known that algal species and communities are sensitive to environmental changes. Species may be either inhibited or stimulated by pollutants. In a community, a pollutant may affect some species but not others, thereby causing changes in species diversity and composition. This can be followed by changes in composition of the animal community and altered routes of flow of energy and materials. Often, the altered ecosystem is undesirable from the human standpoint. On this basis, a bioassay program designed to study effects of suspected pollutants should include research on unicellular algae.

Marine algae tests were performed on 15 textile effluent samples at ERL (Sabine Island, Gulf Breeze, Florida) under the direction of Dr. J. Walsh. Fifteen wastewater samples were subjected to this testing series instead of 23 samples because this bioassay was integrated into the program after sampling began. EPA test procedures (3) used for this analysis were modified as follows:

- Continuous lighting was used instead of a 14-hr-light, 10-hr-dark cycle.
- Salinity was 30 parts per thousand (ppt) instead of 10 ppt.
- Wastes were not sterilized.

- Optical density of cultures was determined on days 3, 4, and 5.
- Final biomass was not estimated.
- Only the 96-hr EC_{50} is reported.

The relationship between optical density as a measure of population density and cell counts was determined by spot checks. In all cases, it was found that optical density and cell counts using a hemocytometer were closely correlated.

In order to estimate stimulation of growth, the ratio of population density in treated samples to population density in controls (T/C) was calculated, and the highest value for each waste is reported.

Effects of the textile wastes on population density of *Skeletonema costatum* are given in Table 34. There was a wide distribution of toxicity. Wastes L and N were by far the most toxic, whereas B, S, U, and X were not toxic.

TABLE 34. RESULTS OF MARINE ALGAE ACUTE TOXICITY TESTS

Plant	Inhibition, EC_{50}, percent secondary effluent	Stimulation: Percent growth over control, T/C	Stimulation: Percent secondary effluent[a]
B	–[b]	200	100
C	90	130	80
E	10 to 50[c]	0	0
F	85	230	10
G	59	130	10
K	77	180	10 to 30
L	1.7	110	0.5
N	2.3	120	0.1 to 0.5
P	9.0	110	0.1
S	–[b]	410	90
T	70	310	35
U	–[b]	180	70
V	94	120	10 to 70
W	50	230	30
X	–[b]	170	95

[a] Percent of secondary effluent solution corresponding to highest stimulation growth rate.

[b] Inhibition less than 50% in 100% secondary effluent.

[c] Small volume of sample received, not enough to complete tests.

Most wastes stimulated growth. Although there were not enough data for proper statistical analysis, wastes with T/C ratios of 1.1 to 1.3 were not significantly different from the controls.

The importance of growth stimulation by waste must not be underestimated. Of the 15 samples, 8 definitely stimulated growth. Five wastes caused population densities two to four times those of the controls.

Another way to look at this is to estimate the concentrations that doubled the population density (T/C equals 2):

Plant	Percent waste
T	<10
S	10
F	30
W	30
B	100

Fewer than 10% of the wastes from Plants U, X, and K causes an increase of 50% in population density (T/C equals 1.5).

Growth stimulation could have a substantial impact on natural bodies of water by causing 1) eutrophication and/or 2) changes in relative numbers of important phytoplankton.

Marine Animal Series

Marine animal bioassay testing was performed to ascertain the concentration of secondary wastewater sample that was acutely toxic to juvenile sheepshead minnows and to grass shrimp. Although none of the textile plants discharge directly into a marine environment, this biotest was performed to provide general information about the toxicity of textile plant wastewaters and to evaluate this new bioassay testing procedure. Since this testing series was integrated into the program after sampling began, only 14 textile plant wastewater samples were subjected to this test.

The EPA static bioassay procedure incorporates several methods (5, 12) and is the simplest, most economic marine animal assay test available. Juvenile sheepshead minnows (*Cyprinodon variegatus*) and adult grass shrimp (*Palaemonetes pugio* or *P. vulgaris*) were used as test animals because they adapt easily to a wide range of salinity and temperature in static bioassays.

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(12) Sprague, J. B. The ABC's of Pollutant Bioassay Using Fish. In: Biological Methods for the Assessment of Water Quality, J. Cairns, Jr., and K. L. Dickson, eds. ASTM Special Technical Publication 528, American Society for Testing and Materials, Philadelphia, Pennsylvania, 1973. pp. 6-30.

Additionally, various phases of their life cycles may be studied due to their short life span.

There are two objections to this procedure: Receiving water characteristics are not closely simulated, and the test organism may not be representative of the most sensitive species in a given geographical area. However, the method is adequate for ranking industries according to their effluent toxicity.

Tests on 14 textile plant wastewater samples were performed at EG&G-Bionomics Marine Research Laboratory (BMRL; Pensacola, Florida) under the direction of Dr. R. Parrish. Dr. J. Walsh was the EPA Technical Advisor associated with the marine ecology biotests.

A total of 0.02 m^3 of secondary effluent sample was collected from each of the 14 plants for sheepshead minnow and grass shrimp acute toxicity analysis. Samples were shipped via air freight in glass bottles, packed in ice, and were stored at BMRL in a room with the temperature controlled at 15 ± 1°C until testing. A description of the samples as they arrived at BMRL is given in Table 35.

TABLE 35. PHYSICAL DESCRIPTION OF EFFLUENT SAMPLES AS THEY ARRIVED AT BMRL FOR SHEEPSHEAD MINNOW AND GRASS SHRIMP ACUTE TOXICITY ANALYSIS

Plant	Description
A	Chlorinated; pH 6.2; cloudy, gray with considerable amount of fine particulate.
B	Nonchlorinated; pH 7.6; clear, light yellow.
C	Nonchlorinated; pH 10.2; clear, blue black with moderate amount of particulate.
E	Nonchlorinated; pH 6.8; clear with small amount of particulate.
F	Nonchlorinated; pH 7.5; clear, light salmon.
G	Chlorinated; pH 6.0; light olive with fair amount of particulate.
K	Chlorinated; pH 7.8; clear, light gray with small amount of particulate.
L	Nonchlorinated; pH 7.3.[a]
N	Nonchlorinated; pH 3.7; clear, light gray with moderate amount of particulate.
S	Nonchlorinated; pH 7.7; clear, light champagne with small amount of particulate.
T	Nonchlorinated; pH 7.4; clear, blue green with moderate amount of particulate.
U	Chlorinated; pH 9.0; clear, dark amber with moderate amount of particulate.
W	Nonchlorinated; pH 8.0; cloudy, orange with moderate amount of particulate.
X	Chlorinated; pH 7.1; clear, light gold with moderate amount of particulate.

[a] Incomplete description because subsample was lost and no other material remained.

Grass shrimp, 15 mm to 26 mm rostrum-telson length, were collected from Big Lagoon, near BMRL. Shrimp were held in flowing water and acclimated to a salinity of 10 ppt for a minimum of 2 days before testing. Mortality was less than 3% during the acclimation.

Methods for the 96-hr, static tests followed EPA test procedures (3) as modified by the EPA Technical Advisor (ERL--Gulf Breeze). Sheepshead minnow and grass shrimp tests were conducted in 0.004-m^3 uncovered glass jars which contained 0.003 m^3 of test solution. Five fish or shrimp were tested per jar, and all test concentrations and controls were duplicated, except as noted. There was no aeration; temperature was maintained at 20 ± 1°C during the tests.

Test concentrations were prepared by mixing appropriate volumes of effluent and dilution water directly in test containers. Dilution water was glass-distilled water adjusted to 10 ppt salinity with Rila Marine Mix (Rila Products, Teaneck, NJ). Batches of dilution water were aged for at least 24 hr with aeration and then filtered (5 μm) before mixing test concentrations. Salinity of the effluents was also adjusted to 10 ppt with Rila Marine Mix before preparing the test concentrations. Control jars received 0.003 m^3 of 10-ppt dilution water, but no effluent.

Range-finding tests (48-hr) were conducted with all effluents to determine appropriate test concentrations for 96-hr definitive tests. Range-finding tests in which no mortality had occurred after 48 hr of exposure to 100% effluent were extended to 96 hr of exposure. If mortality remained less than or equal to 50%, no further tests were conducted with the effluent. In range-finding tests, only five fish or shrimp were tested, and test concentrations were not duplicated.

In cases where mortality was greater than 50% during range-finding tests, 96-hr definitive tests were conducted with five fish or shrimp per jar, and all test concentrations and controls were duplicated.

Based on the test results, 24-hr, 48-hr, and 96-hr LC_{50}'s were graphically interpolated. Two points, representing death in concentrations that were lethal to more than one-half and less than one-half of the fish at the specified times, were plotted on semilogarithmic coordinate paper (test concentrations on the logarithmic axis and corresponding percentages of dead fish on the arithmetic axis, Figure 22). The concentration at which a straight line drawn between the two points crossed the 50% mortality line was the estimated LC_{50}.

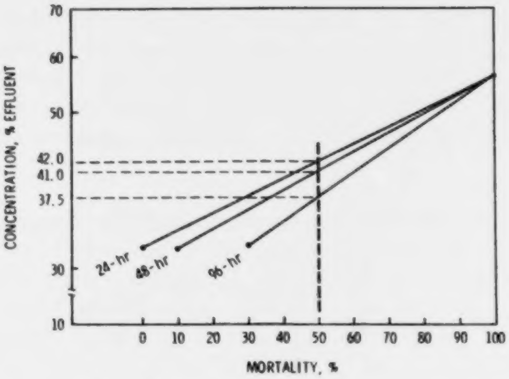

Figure 22. Example of graphically interpolated 24-hr, 48-hr, 96-hr, LC_{50}'s for juvenile sheepshead minnow *(Cyprinodon variegatus)* exposed to textile effluent W.

Sheepshead Minnow Results--

Five of the 14 textile effluents caused greater than 50% mortality of minnows after 96-hr of exposure to effluent concentrations less than or equal to 100% in static, artificial seawater (Table 36). The most toxic effluent (lowest LC_{50} value) was from Plant W, with a graphically interpolated 96-hr LC_{50} of 37.5% (solution containing 37.5% effluent sample). Plant N was less toxic, with a 96-hr LC_{50} of 47.5%. The 96-hr LC_{50}'s for effluents from Plants A, C, and T were 62.0%, 69.5%, and 68.0%, respectively. Four effluents, from Plants E, G, L, and S, caused less than 50% mortality when sheepshead minnows were exposed to 100% effluent for 96-hr. Therefore, no LC_{50}'s were determined for these effluents and values are reported as no acute toxicity. Five effluents, from Plants B, F, K, U, and X, caused no deaths when minnows were exposed to 100% effluent for 96-hr, and 96-hr LC_{50}'s are reported as no acute toxicity effluent.

TABLE 36. ACUTE TOXICITY OF 14 TEXTILE EFFLUENTS TO JUVENILE SHEEPSHEAD MINNOWS *(C. VARIEGATUS)*

	LC_{50}, percent secondary effluent		
Plant	24-Hr	48-Hr	96-Hr
A	75.0	69.5	62.0
B			
C		72.0	69.5
E			
F			
G			
K			
L			
N	75.0	75.0	47.5
S			
T			68.0
U			
W	42.0	41.0	37.5
X			

Note.—Blanks indicate no acute toxicity.

Measured concentrations of dissolved oxygen (DO) in most tests remained greater than 40% of saturation after 96 hr of testing. However, DO was low in test concentrations of Plant T effluent (particularly in 100% effluent) early in the test. Apparently, low DO concentrations in this effluent were due more to the nature of the effluent than to the oxygen demand of test animals.

Grass Shrimp Results--

Five of the 14 textile effluents caused more than 50% mortality of grass shrimp after 96 hr of exposure to effluent concentrations less than or equal to 100% in static, artificial seawater (Table 37). The most toxic effluent was from Plant C, with a graphically interpolated 96-hr LC_{50} of 12.8%. Plant W and A effluents were less toxic, with 96-hr LC_{50}'s of 19.6% and 21.2%; and the 96-hr LC_{50}'s for effluents from Plants N and T were 26.3% and 34.5%, respectively. Three effluents, from Plants E, G, and S, caused less than 50% mortality when grass shrimp were exposed to 100% effluent for 96 hr. Therefore, no LC_{50}'s were determined, and values are reported as no acute toxicity. Six effluents, from Plants B, F, K, L, U, and X, caused no deaths when grass shrimp were exposed to 100% effluent for 96 hr, and 96-hr LC_{50}'s are reported as no acute toxicity.

TABLE 37. ACUTE TOXICITY OF 14 TEXTILE EFFLUENTS TO GRASS SHRIMP (*P. pugio*)

	LC_{50}, percent secondary effluent		
Plant	24-Hr	48-Hr	96-Hr
A	37.6	24.0	21.2
B			
C	17.7	15.5	12.8
E			
F			
G			
K			
L			
N	43.5	37.4	26.3
S			
T			34.5
U			
W	37.5	24.8	19.6
X			

Note.—Blanks indicate no acute toxicity.

Measured concentrations of dissolved oxygen (DO) in most tests remained greater than 40% of saturation after 96 hr of testing. However, DO was low in test concentrations of Plant T effluent (particularly in 100% effluent) early in the test. Apparently, low DO concentrations in this effluent were due more to the nature of the effluent than to the oxygen demand of test animals.

RANGE-FINDING ACUTE TOXICITY 14-DAY RAT TEST

The major objective of any biological testing procedure is the identification of toxicological problems at minimal cost. Therefore, a two-step approach was used to evalute the acute *in vivo* toxicity of samples containing unknown compounds. The first approach is based on the quantal (all-or-none) response; the second is based on the quantitative (graded) response. Normally, the quantal test is used to determine whether or not the quantitative assay is necessary.

The Quantal Test

Five male and five female young adult rats (weighing approximately 250 g each) were purchased from the supplier and conditioned at the laboratory for a minumum of 5 days. A single 10^{-5}-m^3/kg dose of undiluted sample was administered by gavage to each animal. Immediately following administration of the test substance and at frequent intervals during the first day, observations were recorded on all toxic signs or pharmacological effects as described in Table 38 (3). The frequency and severity of the signs were scored. Particular attention was paid to time of onset and disappearance of signs. Daily observations were made on all animals through a 14-day observation period. Effluent samples which produced harmful effects *in vivo* and did not result in deaths were further investigated. At termination of the observation period, all surviving animals were killed and necropsies were performed. Similarly, necropsies were performed on all animals that died during the course of the study.

If mortality did not occur in the quantal study, no further work was done on the test substance, and the LD_{50} was reported as greater than 10 g/kg.

The Quantitative Assay

If a single animal in the quantal study died in the 14-day observation period, a quantitative study was performed. Eighty animals equally divided by sex were maintained for 7 days in quarantine to determine good health in the study population. From these, 40 animals then were randomly divided into 4 groups of 5 male and 5 female animals per group. The test substance, treated as in the quantal test, was administered in graded dosages according to the following schedule: 3.0, 1.0, 0.3, and 0.1 g/kg. Dosage was related to the numbers of animals that died and to the severities and types of signs. Observations,

TABLE 38. PHYSICAL EXAMINATIONS IN ACUTE TOXICITY TESTS IN RODENTS (3)

Organ system	Observation and examination	Common signs of toxicity
Central nervous system and somatomotor	Behavior	Change in attitude to observer, unusual vocalization, restlessness, sedation.
	Movements	Twitch, tremor, ataxia, catatonia, paralysis, convulsion, forced movements.
	Reactivity to various stimuli	Irritability, passivity, anaesthesia, hyperanaesthesia.
	Cerebral and spinal reflexes	Sluggishness, absence.
	Muscle tone	Rigidity, flaccidity.
Autonomic nervous system	Pupil size	Myosis, mydriasis.
	Secretion	Salivation, lacrimation.
Respiratory	Nostrils	Discharge.
	Character and rate of breathing	Bradypnoea, dyspnoea, Cheyne-Stokes breathing, Kussmaul breathing.
Cardiovascular	Palpataion of cardiac region	Thrill, bradycardia, arrhythmia, stronger or weaker beat.
Gastrointestinal	Events	Diarrhea, constipation.
	Abdominal shape	Flatulence, contraction.
	Feces consistency and color	Unformed, black or clay colored.
Genitourinary	Vulva, mammary glands	Swelling.
	Penis	Prolapse.
	Perineal region	Soiled.
Skin and fur	Color, turgor, integrity	Reddening, flaccid skinfold, eruptions, piloerection.
Mucous membranes	Conjunctiva, mouth	Discharge, congestion, hemorrhage cyanosis, jaundice.
Eye	Eyeball	Exophthalmus, nystagmus.
	Transparency	Opacities.
Others	Rectal or pay skin temperature	Subnormal, increased.
	Injection site	Swelling.
	General condition	Abnormal posture, emaciation.

duration of study, and necropsies were carried out as indicated above. The LD_{50} was calculated by the method described in Reference 3.

The range-finding tests were conducted at Litton Bionetics under the direction of Dr. R. Beliles. Dr. J. Stara served as the EPA Technical Advisor.

Actual experimental design parameters used in this study were as follows. Young adult rats of the Charles River CD strain (CRL: COBS CD (SD) BR) were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan. Animals were individually housed in wire bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hr light cycle. Water and Purina Laboratory Chow were provided *ad libitum* with the exception of the night before treatment when food was removed from cages.

Effluent samples were kept refrigerated until used. A single undiluted dose of 10^{-5} m^3/kg of test material was administered by gastric intubation to five rats of each sex. If any rats died at this dose, an LD_{50} value was to be determined by giving additional doses of the test material.

The rats were observed frequently on the day of treatment and daily thereafter. Animals were weighed on the day of treatment, and on days 7 and 14 following treatment. All surviving animals were killed 14 days after treatment and necropsies were performed.

In summary, no rat deaths occurred following the oral administration of 10^{-5} m^3 of effluent sample per kilogram of rat body weight for any of the 23 textile plant effluent samples. Residual effects of treatment were not evident from necropsy findings. Twelve samples (from Plants A, C, E, G, K, M, T, U, V, W, X, and Z) showed no effects that could be related to treatment. Reduced activity was observed immediately after dosing with some of the effluent samples (those from Plants D, H, J, P, and Y). Rats treated with sample from Plant R showed signs of eye irritation which were related to treatment. Effluent samples were collected between the aeration lagoon and the settling pond at Plant R. Reduced body weights or weight gains were noted after administration of five samples (those from Plants B, F, L, N, and S).

A more detailed description of the reactions of the test rats to the effluent samples is given in Appendix E.

SOIL MICROCOSM TEST

Decomposition of dead organic matter by microorganisms is essential for maintaining ecological balance. It is becoming increasingly apparent that as more and more toxic materials from anthropogenic sources are introduced into the soil, they will progressively inhibit these organisms, ultimately creating a critical imbalance.

Soil decomposition is a complex matrix of biological, physical, and chemical processes, dependent on a myriad of variables. Any test designed to detect and ultimately predict the effects of toxic materials on soils must control as many variables as practicable. In addition, disposal of toxic test substances must be simple, convenient, and practical. To meet these needs, a soil/litter microcosm test contained in a "life support" system was designed, constructed, and used to determine the soil response to toxic materials (3).

A soil microcosm is, by design, a miniature model of the natural system--in this case, the site of decomposition in the upper (50 mm) soil layers. In the test microcosm, soil and litter are separately homogenized and layered in an array of airtight containers in which carbon dioxide generation is monitored with time. Carbon dioxide generation is an accepted measure of soil respiration and, in this case, is assumed to be a measure of the microbial activity in the microcosm during the decomposition of organic matter.

Carbon dioxide generation rates were measured from replicates of each waste solution after 2 wk to 3 wk of incubation. For data analysis, rates were compared with three sets of waste solutions and controls using a linear analysis of variance. Treatments showing statistically significant differences in the stimulation of respiration indicated inhibition or stimulation of respiration.

The soil microcosm test was performed at EPA--Corvallis Environmental Research Laboratory, Corvallis, Oregon under the direction of Dr. B. Lighthart. The soil microcosm test used 0.001 m^3 of the 0.01-m^3 freshwater algae samples. The bioassay procedure and apparatus were those described by the EPA (3). A brief description of the materials and methods used is given in Appendix F.

Instead of reporting all data on carbon dioxide (CO_2) generation rates, only the mean qualities of CO_2 produced over the 3-wk incubation period for replicate samples were reported and are presented in Table 39. Samples were analyzed in three batches with one set of replicate controls (using organic-free water) run per batch. The quantity of CO_2 produced each day over the test period was plotted on graph paper and normalized with the control sample. The slope of the resulting curve is the normaalized relative CO_2 rate change (Table 39). If the wastewater sample stimulated the microorganisms, the slope of the curve was positive; if the sample was inhibitory or toxic to the microorganisms, the slope was negative. Of the 23 secondary effluent wastewater samples tested, 15 inhibited the production of CO_2 and 8 stimulated the production of CO_2.

TABLE 39. RESULTS OF SOIL MICROCOSM TESTS ON SECONDARY WASTEWATER SAMPLES

Run	Plant code	Mean total CO_2 produced, m^3	Normalized relative CO_2 rate change[a]	F-value[b]
1	D	221.9×10^{-6}	-0.099	789
	H	220.2×10^{-6}	-0.083	242
	J	211.6×10^{-6}	-0.163	442
	M	229.9×10^{-6}	-0.059	224
	P	238.7×10^{-6}	0.022	11.1
	R[c]	253.3×10^{-6}	-0.062	10.9
	Y	204.3×10^{-6}	-0.172	611
	Z	222.2×10^{-6}	-0.112	465
	Control	246.8×10^{-6}		
2	E	275.9×10^{-6}	-0.048	245
	G	302.0×10^{-6}	0.017	13.5
	K	291.9×10^{-6}	-0.004	33.6
	L	285.5×10^{-6}	-0.020	312
	S	288.4×10^{-6}	-0.017	12.4
	V	275.3×10^{-6}	-0.066	247
	W	305.5×10^{-6}	0.031	12.4
	Control	298.7×10^{-6}		
3	A	246.7×10^{-6}	-0.032	0.14
	B	270.4×10^{-6}	0.020	55.4
	C	285.7×10^{-6}	-0.005	119
	F	236.8×10^{-6}	-0.039	6.15
	N	269.7×10^{-6}	0.059	25.2
	T	276.6×10^{-6}	0.020	62.4
	U	277.3×10^{-6}	0.055	55.0
	X	274.6×10^{-6}	0.047	59.5
	Control	254.2×10^{-6}		

Note.—Blanks indicate information not applicable.

[a] Negative sign indicates inhibition of CO_2 generation rate compared to a control sample; positive sign indicates CO_2 stimulation.

[b] Results are significant at a 95% confidence level for $F > 5.99$ and at a 99% confidence level for $F > 13.7$.

[c] Samples inadvertently collected prior to the settling pond.

The final column in Table 39 as reported by the EPA Technical Advisor is a measure of the statistical significance of the data. A standard "Student t" test was employed using the F-table and all the individual CO_2 generation rate data. Based on those data, if the value of F is greater than 5.99, then the probability of a Type I error is 5%. If the value of F is greater than 13.7, then the probability of a Type I error is 1%. A Type I error means that one rejects the hypothesis when it is in fact true.

From the data in Table 39, all secondary effluent samples have a statistically significant effect at the 5% confidence level. Data for Plant A have a greater than 5% probability of a Type I error. Of the 23 samples, 17 have a statistically significant effect at the 1% confidence level.

SECTION 8

DISCUSSION OF RESULTS

PLANT RANKING BY RELATIVE WASTEWATER TOXICITY

The primary objective of the Phase I screening study was to rank textile plants according to the toxicity of their secondary wastewater and to select plants for detailed toxicity evaluation in Phase II. To accomplish this objective, members of the EPA Bioassay Subcommittee met to evaluate the bioassay data. Members of the Subcommittee are illustrated as EPA Technical Advisors in Figure 20 (Section 7). A summary of all the bioassay results is given in Table 5 (Section 3).

Data evaluation began with ranking of the plants in each set of bioassays. Results are discussed in the following sections.

Freshwater Ecology Series

Results from these tests showed sufficient variation to permit relative ranking of the toxicity of effluent samples. A composite ranking based on the responses of fathead minnows and *Daphnia* is shown in Table 40. No general rule can be made concerning the relative response between fathead minnows and *Daphnia*. For example, Plant E's effluent was significantly toxic to *Daphnia* but not toxic at all to fathead minnows; at Plant T, the reverse was true.

TABLE 40. RELATIVE TOXICITY RANKING BY BIOASSAY TEST

Relative toxicity	Cytotoxicity (RAM)	Composite freshwater ecology (fathead minnow and *Daphnia*)	Composite marine ecology (sheepshead minnows, grass shrimp, and marine algae)	Clonal toxicity (CHO-K1)
Most toxic	C,N	N	L,N,T	D
		A,L		
		C,V		
Intermediate		W		
Toxicity	A,F,L,T,W,X	E	A,C,S,W	M,H,J
		U		
		T		
		G,Z		
Least toxic	M	F,H,M	B,E,F,G,K,U,X	R,P,Y,Z
Nontoxic	B,E,G,K,U,D,H,J,P,R,V,Y,Z	B,K,S,X,D,J,P,Y		
Not analyzed	S		D,H,J,M,P,R,V,Y,Z	A,B,C,E,F,G,K,L,N,S,T,U,V,W,X

At the time of the evaluation, freshwater algae results were not available, but as seen in Table 32 (Section 7), ranking of plants by algal inhibition is similar to that for fathead minnow and *Daphnia*. Because the focus of the program is toxicity removal by treatment technologies and not ecological impacts, algal stimulation effects were not considered in the ranking.

Marine Ecology Series

Based on toxicity data for sheepshead minnows, grass shrimp, and marine algae, ranking of effluents by toxicity was accomplished and is shown in Table 40. In all samples, grass shrimp were more sensitive than sheepshead minnows. Also, the fathead minnows were more sensitive in the majority of the samples than sheepshead minnows. No general correlation was seen between the response of *Daphnia* and grass shrimp.

Cytotoxicity

Rabbit alveolar macrophage tests indicated that none of the samples was highly toxic. Two samples, N and C, were moderately toxic and the following seven samples were slightly toxic: L, W, T, X, A, F, and J.

Only eight samples were tested by MRC using the clonal toxicity test: D, H, J, M, P, R, Y, and Z. Of the eight samples, four showed significant toxicity: D, M, H, and J.

Mutagenicity

None of the 23 effluent samples produced a positive response in any of the bacterial tester strains. The Bioassay Subcommittee expressed concern that the detection limits for this bioassay series were not sensitive enough to detect the presence of significant concentrations (0.001 to 0.1 g/m^3) of chemical mutagens.

Rat Acute Toxicity Tests

No acute toxicity was observed from the maximum dose (10^{-5} m^3/kg) of rat body weight) ingested by the rats. However, six effluent samples showed potential body weight effects: F, N, C, L, S, and B. The subcommittee expressed concern about the detection limits of this test also.

Plant Ranking

Based on all of the above analyses, the subcommittee ranked the 23 textile plants in descending order of secondary effluent toxicity, and results are shown in Table 41.

TABLE 41. PRIORITIZATION OF TEXTILE PLANTS BY TOXICITY OF SECONDARY EFFLUENT

Toxicity ranking	Plant
Most toxic	N,A W C,T V,L
Least toxic	S,P
Nontoxic	B,D,E,F,G,H,J,K M,U,X,Y,Z

From the above list, the subcommittee recommended that the following nine textile plants be tested to determine the removal of toxicity achieved by the tertiary treatment technologies being tested in the ATMI/EPA Grant Study: N, A, L, T, C, P, S, W, and V. (Plant R was also recommended for study under Phase II because its secondary effluent samples were inadvertently collected prior to the settling pond.) In addition, they recommended that the freshwater ecology series be used to measure reduction in wastewater toxicity by the treatment technologies. The marine ecology series was not selected because none of the textile plants discharge wastewater into a marine environment.

PROGRAM OUTLINE FOR PHASE II STUDY

The objective of the second part of the textile wastewater toxicity study is to determine reduction in priority pollutant concentrations and in acute toxicity as a result of applying the ATMI/EPA BATEA tertiary treatment technologies to the secondary effluent at the 10 textile plants.

Pilot plants are scheduled to be at each (10) textile plant for from 6 wk to 8 wk. For the first 4 wk, seven tertiary treatment systems will be tested to determine which one provides the best removal of criteria pollutants. A treatment system consists of one or more of the six tertiary treatment technologies. From the data collected, the "best" system will be identified. This system will then be set up and operated at steady-state conditions for a final period of 2 wk.

For toxicity and priority pollutant analyses at each plant, 24-hr composited samples will be collected during the 2 wk of steady-state operations from the one system identified as the "best available technology." Since the tertiary treatment system will be composed of several of the six treatment technologies, samples will be collected before and after each unit operation in the system, resulting in approximately four samples.

In order to evaluate the reduction in toxicity and priority pollutant concentrations, 24-hr composited secondary effluent samples will also be collected. Due to hydraulic retention time through the pilot plant, secondary effluent sampling will lead the tertiary treatment sampling by the appropriate time for the tertiary treatment system selected.

A 24-hr composited sample of the intake water to the textile plant will be collected at each of the 10 plants to understand the presence of priority pollutants in wastewater samples. Either continuous or grab samples will be collected depending upon the sampling conditions around the intake water facilities. Samples will be collected for volatile organics, nonvolatile organics, and metals analyses. Therefore, a total of approximately 6 samples will be collected at each of the 10 plants as illustrated in Table 42.

TABLE 42. SAMPLE SCHEDULE AT EACH OF THE 10 TEXTILE PLANTS

Sample site	No. of samples	Analyze for
Plant intake water	1	129 priority pollutants
Secondary effluent	1	129 priority pollutants and freshwater ecology series
Best tertiary treatment system	4	129 priority pollutants and freshwater ecology series

The freshwater ecology series consists of bioassay tests on the following three test organisms: fathead minnows, *Daphnia*, and freshwater algae. Five sample fractions are collected for priority pollutant analysis: volatile organics, nonvolatile organics, metals, cyanide, and phenol. Criteria pollutant analyses will not be performed since these analyses will be routinely performed under the ATMI/EPA Grant Study.

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APPENDIX A

RECOMMENDED LIST OF PRIORITY POLLUTANTS

TABLE A-1. RECOMMENDED LIST OF PRIORITY POLLUTANTS

Compound name
Acenaphthene
Acrolein
Acrylonitrile
Benzene
Benzidine
Carbon tetrachloride (tetrachloromethane)
Chlorinated benzenes (other than dichlorobenzenes)
Chlorobenzene
1,2,4-Trichlorobenzene
Hexachlorobenzene
Chlorinated ethanes (including 1,2-dichloroethane, 1,1,1-trichloroethane and hexachloroethane)
1,2-Dichloroethane
1,1,1-Trichloroethane
Hexachloroethane
1,1-Dichloroethane
1,1,2-Trichloroethane
1,1,2,2-Tetrachloroethane
Chloroethane
Chloroalkyl ethers (chloromethyl, chloroethyl and mixed ethers)
Bis(chloromethyl) ether
Bis(2-chloroethyl) ether
2-Chloroethyl vinyl ether (mixed)
Chlorinated naphthalene
2-Chloronaphthalene

(continued)

TABLE A-1 (continued).

Compound name
Chlorinated phenols (other than those listed elsewhere; includes trichlorophenols and chlorinated cresols)
2,4,6-Trichlorophenol
p-Chloro-*m*-cresol (4-chloro-3-methylphenol)
Chloroform (trichloromethane)
2-Chlorophenol
Dichlorobenzenes
1,2-Dichlorobenzene
1,3-Dichlorobenzene
1,4-Dichlorobenzene
Dichlorobenzidine
3,3'-Dichlorobenzidine
Dichloroethylenes (1,1-dichloroethylene and 1,2-dichloroethylene)
1,1-Dichloroethylene (vinylidine chloride)
1,2-*Trans*-dichloroethylene
2,4-Dichlorophenol
Dichloropropane and dichloropropene
1,2-Dichloropropane
1,3-Dichloropropylene (*cis* and *trans*-1,3-dichloropropene)
2,4-Dimethylphenol
Dinitrotoluene
2,4-Dinitrotoluene
2,6-Dinitrotoluene
1,2-Diphenylhydrazine
Ethylbenzene
Fluoranthene

(continued)

TABLE A-1 (continued).

Compound name
Haloethers (other than those listed elsewhere)
4-Chlorophenyl phenyl ether
4-Bromophenyl phenyl ether
Bis(2-chloroisopropyl) ether
Bis(2-chloroethoxy) methane
Halomethanes (other than those listed elsewhere)
Methylene chloride (dichloromethane)
Methyl chloride (chloromethane)
Methyl bromide (bromomethane)
Bromoform (tribromomethane)
Dichlorobromomethane
Trichlorofluoromethane
Dichlorodifluoromethane
Chlorodibromomethane
Hexachlorobutadiene
Hexachlorocyclopentadiene
Isophorone (3,5,5-trimethyl-2-cyclohexen-1-one)
Naphthalene
Nitrobenzene
Nitrophenols (including 2,4-dinitrophenol and dinitrocresol)
2-Nitrophenol
4-Nitrophenol
2,4-Dinitrophenol
4,6-Dinitro-*o*-cresol
Nitrosoamines
N-nitrosodimethylamine
N-nitrosodiphenylamine
N-nitroso-di-n-propylamine
Penta chlorophenol
Phenol

(continued)

TABLE A-1 (continued).

Compound name
Phthalate esters
Bis(2-ethylhexyl) phthalate
Butyl benzyl phthalate
Di-n-butyl phthalate
Diethyl phthalate
Dimethyl phthalate
Di-n-octyl phthalate
Polynuclear aromatic hydrocarbons
Benz(a)anthracene (1,2-benzanthracene)
Benzo(a)pyrene (3,4-benzopyrene)
3,4-Benzofluoranthene
Benzo(k)fluoranthene (11,12-benzofluoranthene)
Chrysene
Acenaphthylene
Anthracene
Benzo(ghi)perylene (1,12-benzoperylene)
Fluorene
Phenanthrene
Dibenz(ah)anthracene (1,2,5,6-dibenzanthracene)
Indeno(1,2,3-cd)pyrene (2,3-*o*-phenylenepyrene)
Pyrene
Tetrachloroethylene
Toluene
Trichloroethylene
Vinyl chloride (chloroethylene)
Pesticides and metabolites
Aldrin
Dieldrin
Chlorodane (technical mixture and metabolites)
DDT and metabolites
4,4'-DDT
4,4'-DDE (p,p'-DDX)
4,4'-DDD (p,p'-TDE)

(continued)

TABLE A-1 (continued).

Compound name
Endosulfan and metabolites
α-Endosulfan
β-Endosulfan
Endosulfan sulfate
Endrin and metabolites
Endrin
Endrin aldehyde
Heptachlor and metabolites
Heptachlor
Heptachlor epoxide
Hexachlorocyclohexane
α-BHC
β-BHC
λ-BHC (lindane)
δ-BHC
Polychlorinated biphenyls (PCB)
PCB-1242 (Arochlor 1242)
PCB-1254 (Arochlor 1254)
PCB-1221 (Arochlor 1221)
PCB-1232 (Arochlor 1232)
PCB-1248 (Arochlor 1248)
PCB-1260 (Arochlor 1260)
PCB-1016 (Arochlor 1016)
Toxaphene
Elements
Antimony (Total)
Arsenic (Total)
Asbestos (Fibrous)
Beryllium (Total)
Cadmium (Total)
Chromium (Total)
Copper (Total)
Cyanide (Total)
Lead (Total)

(continued)

TABLE A-1 (continued).

Compound name
Elements (continued)
Mercury (Total)
Nickel (Total)
Selenium (Total)
Silver (Total)
Thallium (Total)
Zinc (Total)
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)

APPENDIX B

PRIORITY POLLUTANT ANALYSIS FRACTIONS

TABLE B-1. VOLATILE COMPOUNDS

Compound	Compound
Chloromethane	1,2-Dichloropropane
Dichlorodifluoromethane	*trans*-1,3-dichloropropene
Bromomethane	Trichloroethylene
Vinyl chloride	Dibromochloromethane
Chloroethane	*Cis*-1,3-dichloropropene
Methylene chloride	1,1,2-Trichloroethane
Trichlorofluoromethane	Benzene
1,1,-Dichloroethylene	2-Chloroethyl vinyl ether
1,1-Dichloroethane	Bromoform
trans-1,2,-dichloroethane	1,1,2,2-Tetrachloroethylene
Chloroform	1,1,2,2-Tetrachloroethane
1,2-Dichloroethane	Toluene
1,1,1-Trichloroethane	Chlorobenzene
Carbon tetrachloride	Ethylbenzene
Bromodichloromethane	Acrolein
Bis(chloromethyl) ether	Acrylonitrile

TABLE B-2. BASE NEUTRAL EXTRACTABLE COMPOUNDS

Compound	Compound
1,3-Dichlorobenzene	Anthracene
1,4-Dichlorobenzene	Diethyl phthalate
Hexachloroethane	Dimethyl phthalate
1,2-Dichlorobenzene	Fluoranthene
Bis(2-chloroisopropyl) ether	Pyrene
Hexachlorobutadiene	Di-n-butyl phthalate
1,2,4-Trichlorobenzene	Benzidine
Naphthalene	Butyl benzyl phthalate
Bis(2-chloroethyl) ether	Chrysene
Hexachlorocyclopentadiene	Bis(2-ethylhexyl) phthalate
Nitrobenzene	Benz(a)anthracene
Bis(2-chloroethoxy) methane	Benzo(b)fluoranthene
2-Chloronaphthalene	Benzo(k)fluoranthene
Acenaphthylene	Benzo(a)pyrene
Acenaphthene	Indeno(1,2,3-cd)pyrene
Isophorone	Dibenz(a,h)anthracene
Fluorene	Benzo(g,h,i)perylene
2,6-Dinitrotoluene	N-nitrosodimethylamine
1,2-Diphenylhydrazine	N-nitroso-di-n-propylamine
2,4-Dinitrotoluene	4-Chlorophenyl phenyl ether
N-nitrosodiphenylamine	3,3'-Dichlorobenzidine
Hexachlorobenzene	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin[a]
4-Bromophenyl phenyl ether	
Phenanthrene	Bis-(chloromethyl) ether

[a] This compound was specifically listed in the consent decree. Because of TCDD's extreme toxicity, EPA recommends that laboratories not acquire analytical standards for this compound.

TABLE B-3. ACID EXTRACTABLE COMPOUNDS

2-Chlorophenol
Phenol
2,4-Dichlorophenol
2-Nitrophenol
p-Chloro-*m*-cresol
2,4,6-Trichlorophenol
2,4-Dimethylphenol
2,4-Dinitrophenol
4,6-Dinitro-*o*-cresol
4-Nitrophenol
Pentachlorophenol

TABLE B-4. PESTICIDES AND PCB

Compound
β-Endosulfan
α-BHC
γ-BHC
β-BHC
Aldrin
Heptachlor
Heptachlor epoxide
α-Endosulfan
Dieldrin
4,4'-DDE
4,4'-DDD
4,4'-DDT
Endrin
Endosulfan sulfate
δ-BHC
Chlordane
Toxaphene
PCB-1242 (Aroclor 1242)
PCB-1254 (Aroclor 1254)
PCB-1221 (Aroclor 1221)
PCB-1232 (Aroclor 1232)
PCB-1248 (Aroclor 1248)
PCB-1260 (Aroclor 1260)
PCB-1016 (Aroclor 1016)

TABLE B-5. METALS AND OTHER COMPOUNDS

Metals, total	Others
Antimony	Asbestos
Arsenic	Cyanide
Beryllium	
Cadmium	
Chromium	
Copper	
Lead	
Mercury	
Nickel	
Selenium	
Silver	
Thallium	
Zinc	

APPENDIX C

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APPENDIX D

REACTION OF FATHEAD MINNOWS AND DAPHNIA TO TEXTILE SECONDARY EFFLUENTS

Plant A

This sample was acutely toxic to both the fathead minnow and *D. pulex*, resulting in estimated 96-hr LC_{50} and 48-hr EC_{50} values of 19.0% and 9.0% effluent dilution, respectively. The 2-day delay from collection of the sample to the beginning of the test probably did not affect sample toxicity.

Control fish ranged in length 30 mm to 39 mm, with the average being 34.1 mm. Their weight ranged from 0.2 g to 0.6 g, with the average being 0.37 g.

Plant B

This sample was not acutely toxic to either the fathead minnow or *D. pulex*.

Control fish ranged in length from 25 mm to 51 mm, with the average being 36.5 mm. Their weight ranged from 0.2 g to 1.25 g, with the average being 0.56 g.

Plant C

This sample was acutely toxic to both fathead minnow and *D. pulex*, resulting in estimated 96-hr LC_{50} and 48-hr EC_{50} values of 46.5% and 41.0% effluent dilution, respectively. All fish died in 100% effluent, and half of the fish died in 60% effluent dilution within the first 1/2 hr. The remaining fish in the 60% dilution were dead by the next morning.

Control fish ranged in length from 28 mm to 38 mm, with the average being 33.5 mm. Their weight ranged from 0.2 g to 0.5 g, with the average being 0.30 g.

Plant D

At the end of the test, fish from the control were measured and weighed. Fish ranged in length from 32 mm to 48 mm, with an average 38.6 mm. Fish ranged in weight from 0.15 g to 0.73 g, with an average weight of 0.34 g. At the end of the test, two fish had died in the 60% dilution and one in the 4.7% dilution. In the *D. pulex* test, no animals died during the 48-hr period.

Results indicate that this sample was not acutely toxic to fathead minnows or *D. pulex*.

Plant E

At the end of the test, fathead minnows from the control jar were measured and weighed. Fish ranged in length from 21 mm to 37 mm, with an average length of 31 mm. Their weight ranged from 0.1 g to 0.4 g, with the average being 0.29 g.

Data indicate that this textile mill sample was not acutely toxic to fathead minnows over a 96-hr period. However, the sample was acutely toxic to the *D. pulex*, with an estimated 48-hr EC_{50} of 7.8 % waste.

Plant F

This sample evidenced no acute 96-hr toxicity to fathead minnows. However, the estimated 48-hr EC_{50} value of 81.7% indicated the sample was somewhat toxic to *D. pulex*.

Fish ranged in length from 28 mm to 38 mm, with the average being 32.1 mm. Their weight ranged from 0.2 g to 0.5 g, with the average being 0.28 g.

Plant G

This sample indicated acute toxicity to both fathead minnow and *D. pulex* with estimated 96-hr LC_{50} and 48-hr EC_{50} values of 64.7% and 62.4% effluent dilution, respectively. In this instance, toxicity was essentially the same for both species.

Fish from the control jar ranged in length from 26 mm to 35 mm, with an average length of 29.6 mm. Their weight ranged from 0.15 g to 0.40 g, with the average being 0.26 g.

Plant H

The fathead minnow bioassay is inconclusive for this sample. It appears that the fish were diseased. There was considerable mortality in all of the jars including the control. This was not a reliable test run.

The bioassay run using the *D. pulex*, however, was meaningful. Mortality in the 100% effluent was 40%. In the other concentrations, 10 *D. pulex* were alive at the end of the test. The 100% concentration of effluent showed some indication of toxicity, although an EC_{50} value could not be determined.

Plant J

Fish from the control jar were weighed and measured at the end of the test. Fish ranged in length from 32 mm to 44 mm, with an average of 37 mm. They ranged in weight from 0.3 g to 0.75 g, with an average weight of 0.46 g. At the end of the test, one fish had died in the 100%, one in the 60%, two in the 7.8%, and three in the 13% dilutions. Three of the *D. pulex* died in 100% effluent; one died in the 60% dilution; and one died in the control jar.

Based on the results from these test, this sample was not acutely toxic to the fathead minnows or the *D. pulex*.

Plant K

This sample indicated no acute toxicity to fathead minnows and little toxicity to *D. pulex*. Four *D. pulex* died in 100% effluent during the 48-hr period of the test.

Control fish ranged in length from 28 mm to 35 mm, with an average length of 30 mm. Their weight ranged from 0.15 g to 0.4 g, with the average being 0.26 g.

Plant L

This sample was quite acutely toxic to both fathead minnows and *D. pulex*, resulting in estimated 96-hr LC_{50} and 48-hr EC_{50} values of 23.5% and 28.0% effluent dilution, respectively. The two statistical procedures used could not be utilized to calculate the toxicity values with 95% confidence limits for the *D. pulex*.

Control fish ranged in length from 26 mm to 40 mm, with the average being 30.6 mm. Their weight ranged from 0.2 g to 0.6 g, with the average being 0.33 g.

Plant M

At the end of the test, fathead minnows in the control jar weighed an average 0.29 g, with a range of 0.15 g to 0.50 g. Average length of the fish was 34 mm, with a range of 28 mm to 48 mm.

Data indicate that this sample was not acutely toxic to the fathead minnow, but it was acutely toxic to *D. pulex*. A 48-hr EC_{50} value of 60.0% effluent, with 95% confidence limits of 40.72% to 88.95%, was determined with the moving average angle procedure.

Plant N

This sample was acutely toxic to both fathead minnow and *D. pulex*. The estimated 96-hr LC_{50} value for the fish was 48.8% effluent dilution. All fish in 100% effluent were dead within 19 hr after the beginning of the test. The fish showed evidence of hemorrhaging aroung the mouth and tail. The pH of the 100% effluent was 4.0 at the beginning of the test and only 4.5 24 hr later. Thus, low pH was responsible for this mortality. The sample was extremely toxic to *D. pulex*, with all animals being killed within 24 hr in as low as 13% effluent dilution, and all were dead in every dilution at the end of 48 hr. The 2-day delay from the time of collection to the beginning of the test probably did not affect the toxicity. The temperature of the sample was 2°C when it reached the Newtown Fish Toxicology Station. Biological degradation would be minimal under these conditions.

Control fish ranged in length from 28 mm to 45 mm, with the average being 36.2 mm. Their weight ranged from 0.2 g to 1.0 g, with the average being 0.55 g.

Plant P

At the end of the test (96 hr), there were nine fish surviving in each test jar. None of the fish in the control jar died. The sample did not indicate 96-hr acute toxicity.

At the end of the test there wer 10 *Daphnia* alive in each container.

Results indicate that this sample was not acutely toxic to either fathead minnows or *D. pulex*.

Plant R

Based on appearance, this sample, was both high in suspended solids and highly turbid (149 units). Turbidity of previous samples ranged from a low of 6 units to a high of 54 units. Considerable sample agitation achieved only 2.8 g/m^3 dissolved oxygen in the 100% effluent at the beginning of the test.

At the end of the test, fish from the control jar averaged 0.28 g in weight, with a range of 0.15 g to 0.65 g. Average length was 35.4 mm, with a range of 28 mm to 47 mm.

Mortality in both the fathead minnow bioassay test and the *D. pulex* test usually was observed within the first 24 hr. BOD appears to have been a contributing factor. Dissolved oxygen dropped to low levels in the four high effluent volumes. However, after 24 hr, dissolved oxygen was still at a level (0.3 g/m^3 to 1.8 g/m^3) wherein fathead minnows can survive for a considerable period of time. In 18 hr there was a complete kill of the fish in 100% and 60% effluent. In 36% and 21.6% effluent there was 80% and 30% mortality, respectively, in 24 hr.

Probit analysis of the data at the end of the fathead bioassay (96 hr) indicates an LC_{50} value of 16.45% effluent, with 95% confidence limits of 12.39% to 21.72%. A 48-hr LC_{50} value for *D. pulex* could not be determined with probit analysis. The moving average angle procedure indicated the 48-hr EC_{50} value to be 7.96% effluent, with 95% confidence limits of 6.14% to 7.96%. Thus, the data show this particular textile mill effluent sample to be acutely toxic to both fathead minnows and *D. pulex*. The *D. pulex* were more sensitive than the fathead minnows.

Plant S

This sample exhibited no 96-hr acute toxicity to fathead minnows and little toxicity to *D. pulex*. There were three, two, and one *D. pulex* deaths in 100%, 60%, and 36% effluent dilution, respectively.

Unlike all other samples, this sample contained a heavy, flocculent, fibrous material that settled to the bottom of the test containers (to a depth approximately 5.1 cm from the 100% effluent container). Fish hid in this material throughout the test and were not adversely affected.

Control fish ranged in length from 27 mm to 34 mm, with the average being 29.6 mm. Their weight ranged from 0.20 g to 0.35 g, with the average being 0.25 g.

Plant T

This sample was acutely toxic to fathead minnow, with an estimated 96-hr LC_{50} value of 46.5% effluent dilution. All of the fish were dead in 100% effluent within 18 hr after the test was started. Slight evidence of toxicity to *D. pulex* was indicated with three animals dying in 100% effluent and one in the 60% dilution. The 2-day delay from the time of collection to the beginning of the test probably did not affect the toxicity of the sample.

Control fish ranged in length from 26 mm to 38 mm, with the average being 31.3 mm. Their weight ranged from 0.2 g to 0.5 g, with the average being 0.33 g.

Plant U

This sample was not acutely toxic in 96 hr to fathead minnow. However, it was toxic to *D. pulex*, having an estimated 48-hr EC_{50} value of 12.1% effluent dilution. The 2-day delay from sample collection to the beginning of the test probably did not affect its toxicity.

The length of the control fish ranged from 29 mm to 46 mm, with the average being 37.7 mm. Their weight ranged from 0.2 g to 1.0 g, with the average being 0.51 g.

Plant V

This sample indicated acute toxicity to both fathead minnows and *D. pulex*. All fish died in 100% effluent within 1 hr; all died in the 60% dilution within 2.5 hr. The estimated 96-hr LC_{50} was 36% effluent dilution. *D. pulex* were much more sensitive with an estimated EC_{50} value of 9.4% effluent.

Fathead minnows in the control jar at the end of the test ranged in length from 27 mm to 38 mm, with an average length of 29 mm. Their weight ranged from 0.1 g to 0.45 g, with an average weight of 0.23 g.

Plant W

This sample was acutely toxic to fathead minnow and *D. pulex*, resulting in estimated 96-hr LC_{50} and 48-hr EC_{50} values of 55.2% and 6.3% effluent dilution, respectively. The sample was much more toxic to *D. pulex* than to fathead minnow.

Control fish ranged in length from 28 mm to 41 mm, with the average being 34 mm. Their weight ranged from 0.2 g to 0.7 g with the average being 0.37 g.

Plant X

This sample was not acutely toxic to either fathead minnows or *D. pulex*. In contrast with a 2-day delay for most samples, it should be noted that there was a delay of 2 day from the time of sample collection until the test was begun. It is possible that the toxic components may have decomposed during the extra day of storage, although this seems unlikely when compared to other samples.

Control fish ranged in length from 28 mm to 42 mm, with the average being 35.3 mm. Their weight ranged from 0.2 g to 0.85 g, with the average being 0.48 g.

Plant Y

The fathead minnow bioassay was not significant with this sample because a disease caused the loss of 30% of the control fish. Despite the disease problem, there was 70% to 90% survival of the fish throughout the range of effluent dilutions. In the

D. pulex test, only one animal died in 100% effluent and one in the 21.6% dilution.

Based on the results from these tests, this sample was not acutely toxic to fathead minnows or *D. pulex*.

Plant Z

At the end of the test, fathead minnows in the control jar averaged 0.5 g in weight with a range of 0.2 g to 1.1 g. Average length of the fish was 35.8 mm, with a range of 28 mm to 50 mm.

Data indicate that this particular sample was not acutely toxic to fathead minnow. However, the sample was acutely toxic to *D. pulex*. A 48-hr EC_{50} value of 42.57% with 95% confidence limits of 30.79% to 64.07% was determined with probit analysis.

APPENDIX E

REACTIONS OF RATS TO TEXTILE PLANT SECONDARY EFFLUENT

Plant A

Although reduced activity was observed immediately after treatment, there were no deaths. Necropsy showed mottled kidneys; however, this has been observed frequently in untreated rats in the Litton Bionetics laboratory and was not considered treatment related. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	240	312	340
	F	240	250	266

Data did not suggest any treatment effect.

Plant B

There were no deaths following treatment. One male had soft stools during several days of the observation period. Necropsy findings were limited to kidney changes described above. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	249	248	300
	F	202	233	241

Body weight loss of males between 0 and 7 was not normal.

Plant C

There were no deaths and no signs of toxicity following treatment. Necropsy findings were limited to kidney changes previously described. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	297	328	365
	F	214	221	223

Data did not suggest any treatment-related effect.

Plant D

There were no deaths following treatment. Reduced activity immediately after treatment among all male rats and a soft stool in one male rat 1 day after treatment were observed. Necropsy showed mottled kidneys; however, this was not considered treatment related. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5}m^3/kg	M	164	235	274
	F	171	208	216

Body weight data did not suggest any adverse effect.

Plant E

There were no deaths following treatment. Signs of eye irritation appeared in 6 of 10 rats near the end of the 14-day observation period, but were not considered treatment related. Necropsy findings, limited to changes in heart surface and kidneys, were observed only among male rats; they also were not considered treatment related. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	176	225	283
	F	157	175	214

Body weight data did not suggest any treatment effect.

Plant F

There were no deaths and no signs of toxicity following treatment. Necropsy findings were limited to kidney changes previously described. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	187	244	291
	F	210	235	229

Decreased body weights were observed in four of five females during the last 7 days of the observation period.

Plant G

There were no deaths following treatment. Signs of eye irritation were observed in a few rats near the end of the observation period. Because of the time elapsing between treatment and the onset of changes, no treatment relationship was judged to be present. Necropsy findings were limited to heart and kidney changes previously described. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	171	212	263
	F	166	173	214

Body weight data did not suggest any treatment effect.

Plant H

Although reduced activity was observed among the females immediately following treatment, there were no deaths. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	198	251	268
	F	180	224	229

Body weight data did not suggest any treatment related effect.

Plant J

Although reduced activity was observed immediately after treatment, there were no deaths. Mottled kidneys were observed at necropsy, but were not considered treatment related. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	201	275	310
	F	187	221	232

Body weight data did not suggest any adverse effect.

Plant K

There were no deaths following treatment. Signs were limited to redness around the eyes of one female rat. Necropsy findings were limited to mottled kidneys. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	209	277	313
	F	192	233	248

Body weight data did not suggest any treatment effect.

Plant L

There were no deaths and no signs of toxicity following treatment. Necropsy findings were limited to kidney changes previously described among the male rats. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	335	354	380
	F	238	240	251

Weight gain among female rats was less than normal.

Plant M

There were no deaths and no signs of toxicity except for soft stool in one male rat on day 11 after treatment. Necropsy findings were limited to mottled kidneys. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	262	320	332
	F	196	227	233

Data did not suggest any treatment-related effect.

Plant N

There were no deaths and no signs of toxicity following treatment. Except for kidney changes previously described, Necropsy findings were limited to a distended cecum in one male rat. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	166	267	284
	F	193	237	213

Loss of body weight among females between day 7 and 14 was unusual.

Plant P

There are no deaths following treatment. Soft stools were observed in one male rat following treatment. Necropsy findings were limited to kidney changes previously described. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	164	239	284
	F	170	204	212

Body weight data did not suggest any adverse effect.

Plant R

There were no deaths following treatment. Dark red material was observed around the nose or eyes of male rats the day following treatment. No other signs were observed. Necropsy findings consisted of several instances of mottled kidneys. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	236	292	320
	F	196	225	231

Body weight data did not suggest any treatment related effect.

Plant S

There were no deaths following treatment. Redness around the eye of one male rat seen near the end of the observation period was not judged to be related to the treatment. Necropsy findings were limited to kidney changes previously described. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	312	341	364
	F	237	239	248

Weight gain of females in the first seven days after dosing was judged to be below normal.

Plant T

There were no deaths and no signs of toxicity following treatment. Necropsy showed mottled kidneys in only one rat. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	172	268	289
	F	208	234	249

Data did not suggest any treatment-related effect.

Plant U

There were no deaths and no signs of toxicity following treatment. Necropsy findings were limited to kidney changes previously described. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	360	403	435
	F	206	231	239

Data did not suggest any treatment-related effect.

Plant V

There were no deaths following treatment. Signs suggestive of eye irritation occurred in 4 of 10 treated rats. These appeared 4 to 5 days after administration of test material and were not judged to be related to treatment. Except for mottled kidneys, necropsy signs were limited to rough appearance of heart ventricles. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	174	233	270
	F	158	171	208

Body weight data did not suggest any adverse effect.

Plant W

There were no deaths and no signs of toxicity following treatment. Necropsy findings were limited to kidney changes previously described among male rats. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	350	378	394
	F	226	234	244

Data did not suggest any treatment-related effect.

Plant X

There were no deaths and no signs of toxicity following treatment. Necropsy findings were limited to kidney changes previously described. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	Day 7	Day 14
10^{-5} m^3/kg	M	291	359	374
	F	203	235	247

Data did not suggest any treatment-related effect.

Plant Y

Although reduced activity was observed immediately following treatment, there were no deaths. One female rat developed an ulceration on the ventral thorax 9 days after treatment, but this was not considered treatment related. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	 7	 14
10^{-5} m^3/kg	M	220	277	318
	F	191	214	233

Body weight data did not suggest any treatment-related effect.

Plant Z

There were no deaths and no signs of toxicity following treatment. Except for mottled kidney changes, Necropsy findings were limited to an unusual rough appearance of the right ventricle in one male rat. Mean body weights (grams) are tabulated below.

Dose	Sex	Day 0	 7	 14
10^{-5} m^3/kg	M	249	311	339
	F	198	228	236

Data did not suggest any adverse effect.

APPENDIX F

PROTOCOL TO TEST EFFECTS OF WASTE MATERIALS ON MICROBIAL RESPIRATION (CARBON DIOXIDE REDUCTION) IN A SIMPLE SOIL MICROCOSM

Materials:

- Homogenized soil from site in question
- 12 - 9.46 x 10^{-4} m^3 Mason jars with airtight lids
- 12 - 3 x 10^{-5} m^3 carbon dioxide trap bottles with airtight lids
- 20°C incubator
- Approximately 0.5 N NaOH solution
- Approximately 0.6 N HCl solution
- 8 - bent glass rods
- 10 x 10^{-6} m^3 burette or titrometric device
- Trizma (to prepare standard)

Methods:

(A) Soil preparation:

- Air dry soil and grind to pass a 1- to 2-mesh screen. Ball milling or crushing may be required to break larger particles.

(B) Microcosm preparation:

- Weigh 100 g (air dried) sieved and homogenized soil into each of eight Mason jars. One set of four replicates each for test and control treatments.

- Optional soil inoculum solution:
  - Thoroughly mix approximately 200 g of fresh nondried soil with 10^{-3} m^3 water.
  - Separate microorganisms from sediment by filtration or light centrifugation.

- Moisten soil in each Mason jar from 60% to 80% of field water holding capacity (FWHC) by uniformly pipetting dropwise over the surface in each of four replicates, 25 x 10^{-6} m^3 of either test or control solution, plus 5 x 10^{-6} m^3 of inoculum and/or water to bring soil to desired FWHC.

GLOSSARY

acute toxicity: Toxic effects to an organism due to a short-term exposure.

concentration: Amount of sample (or toxicant) by weight or volume per unit volume of test solution.

criteria pollutants: Pollutant species identified by EPA - Effluent Guidelines Division which require effluent standards and include BOD_5 COD, TSS, chrome, phenol, color, sulfide, and pH for the textile industry.

cytotoxicity: Toxicity to mammalian cells.

dose: Measured weight or volume of sample (or toxicant) fed to test organism.

EC_{50}: Effective concentration at which 50% of the test organisms reach the desired effect. The "effect", for example, can be growth inhibition or stimulation.

gastrointubation: Insertion of a tube into the intestinal tract to feed effluent sample to test animal.

gavage: Forced feeding of an animal through a tube.

hemocytometer: Microscope slide with square rulings used for counting blood corpuscles or other cells.

in vitro: Describing a biological reaction which can be performed outside the living organism, such as in a test tube.

in vivo: Describing a biological reaction which takes place within the living organism.

LC_{50}: Lethal concentration fifty - calculated concentration of substance which is expected to cause death in 50% of the test organism population, as determined from their exposure to the substance.

LD_{50}: Lethal dose fifty - calculated dose of chemical substance which is expected to cause death in 50% of the test organism population, as determined from their exposure to the substance.

moving average angle: Iterative computer model designed to estimate the median of a tolerance distribution using number of test species used and number that dried due to exposure to the sample.

necropsy: Sacrificing the test organism to perform an autopsy.

priority pollutants: The 129 chemical species identified by EPA as a result of the consent decree.

probit analysis: Iterative computer model designed to calculate LC_{50} values from dose response tests using dosage values, number of test species in control and those exposed to the effluent sample, and probability values of response.

raw waste: Untreated wastewater as it leaves the textile plant and enters the wastewater treatment facility.

secondary effluent: Textile wastewater treated by aerated lagoons and clarified.

soil microcosm: Miniature model of the natural system; in this case, the site of decomposition in the upper (5 cm) soil layer.

viability: Capacity of an organism to live and grow.

CONVERSION FACTORS AND METRIC PREFIXES (13)

CONVERSION FACTORS

To convert from	to	Multiply by
Grams/meter^3 (g/m^3)	Milligrams/liter	1.0
Kilogram (kg)	Pound-mass (avoirdupois)	2.205
Meter (m)	Inch	3.937×10^1
Meter^3 (m^3)	Gallon (U.S. liquid)	2.642×10^2
Meter^3 (m^3)	Liter	1.0×10^3

METRIC PREFIXES

Prefix	Symbol	Multiplication factor	Example
Kilo	k	10^3	5 kg = 5×10^3 grams
Milli	m	10^{-3}	5 mg = 5×10^{-3} gram
Micro	μ	10^{-6}	5 μg = 5×10^{-3} gram

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(13) Standard for Metric Practice. ANSI/ASTM Designation: E 380-76$^{\varepsilon}$, IEEE Std 268-1976, American Society for Testing and Materials, Philadelphia, Pennsylvania, February 1976. 37 pp.